UNIVERSITY OF ALBERTA

Studies on the efficiency of rapeseed (*Brassica napus* L.) germplasm for the acquisition and the utilization of inorganic nitrogen

BY

Juan Sergio Moroni ©

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy

IN

Plant Breeding

DEPARTMENT OF PLANT SCIENCE

EDMONTON, ALBERTA
FALL, 1997

National Library
of Canada

Acquisitions and
Bibliographic Services

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale
du Canada

Acquisitions et
services bibliographiques

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-23040-6

Canada

UNIVERSITY OF ALBERTA
LIBRARY RELEASE FORM

NAME OF AUTHOR: **Juan Sergio Moroni**

TITLE OF THESIS: **Studies on the efficiency of rapeseed (*Brassica napus* L.) germplasm for the acquisition and the utilization of inorganic nitrogen**

DEGREE: **Doctor of Philosophy**

YEAR THIS DEGREE GRANTED: **1997**

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permision.

JS Moroni
....
Juan Sergio Moroni

Apt. 104, 10745-79 Ave.
Edmonton, Alberta
Canada, T6E 1S4

Date: September 26, 1997.

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Studies on the efficiency of rapeseed (*Brassica napus* L.) germplasm for the acquisition and the utilization of inorganic nitrogen**, submitted by **Juan Sergio Moroni** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Plant Breeding.**

Dave Hume

Dr. Dave J. Hume
(External examiner)

Dr. Gary R. Stringam
(Supervisor)

Dr. Jane R. King

Dr. Gregory J. Taylor

Dr. Keith G. Briggs

Date: 24 September 1997.

"For as knowledges are now delivered, there is a kind of contract of error between the deliverer and the receiver; for he that delivereth knowledge desireth to deliver it in such form as may be best believed, and not as may be best examined; and he that receiveth knowledge desireth rather present satisfaction than expectant inquiry"

Sir Francis Bacon
The Advancement of Learning, 1605

"Why pick Cerion? Why, indeed, spend so much time on any detailed particular when all the giddy generalities of evolutionary theory beg for study in a lifetime too short to manage but a few? Iconoclast that I am, I would not abandon the central wisdom of natural history from its inception -that concepts without percepts are empty (as Kant said), and that no scientist can develop an adequate "feel" for nature (that undefinable prerequisite of true understanding) without probing deeply into minute empirical details of some well-chosen group of organisms. Thus, Aristotle dissected squids and proclaimed the world's eternity, while Darwin wrote four volumes on barnacles and on the origin of species"

Stephen Jay Gould
The Flamingo's Smile: Reflections in Natural History
W. W. Norton & Company, Inc., New York. 1985

*To **Jonathan** and **Chantelle**,*
may they be blessed
to experience
the wonders of nature

Abstract

The utilization of rapeseed (*Brassica napus* L.) cultivars with improved N-efficiency may ameliorate the negative impact of N-fertilizers in the environment. The objectives of this research were: (1) to determine whether there is genetic variation for N-efficiency amongst germplasm accessions of *B. napus*, (2) to determine if the genetic variation for N-efficiency is potentially useful in a breeding program and, (3) to obtain information on the genetic nature of N-uptake and N-utilization efficiency of *B. napus*. Response of *B. napus* late in the rosette stage to one N-level (static efficiency) or to increased N-levels (response-rate efficiency) were used as definitions of N-efficiency. Studies carried out to develop a screening technique for selecting N-efficient *B. napus* indicated that growth of *B. napus* was maximized when some N was supplied as NH_4^+ and that the shoot system, and not the root system, was a major factor in determining N-efficiency components in *B. napus* genotypes. Furthermore, studies of the time-course responses to N supply and of the effect of N supply on *B. napus* growth and N-efficiency components late in the rosette stage provided information for selecting sampling time, N-treatment levels, and N efficiency parameters for screening N efficient germplasm.

A screening of 112 genotypes under deficient and sufficient N levels indicated a large variability for both static and response-rate N efficiencies. However, the largest range of variability amongst genotypes was shown for the nitrogen use efficiency (NUE) parameter. A screening of a doubled haploid population derived from parents differing in N-efficiency indicated a large genetic component coupled with a strong maternal effect for many of the N-efficiency parameters.

Nitrogen use efficiency appeared to be the N efficiency parameter with the most potential for breeding in *B. napus*. However, this was accompanied by a strong environmental effect. It can be concluded that: (1) genetic variation for N-efficiency

parameters amongst germplasm accessions of *B. napus* is available; (2) the genotypic variation might be potentially useful in a breeding program; (3) there is a large genetic component for N-efficiency coupled with an strong maternal effect; (4) a static N efficiency parameter such as NUE is potentially the most promising for crop improvement and (5) breeding of N-efficient *B. napus* germplasm might be possible, but difficult.

Acknowledgments

I would like to thank Dr. Gary Stringam for the opportunity to carry out this research, for his support and for reviewing this manuscript. I would also like to express my gratitude to Dr. Mohan Thiagaraja, Dr. Gregory Taylor and Dr. Jane King for their advice, encouragement and support throughout the performance of this research, as well as for reviewing this manuscript.

I would also like to thank Dr. Dave Hume and Dr. Keith Briggs for participating in my defense and reviewing this manuscript. Advice on statistical analysis provided by Dr. Peter Blenis and Dr. Henry Kleingebink, and on greenhouse culture by Bruce Alexander are sincerely appreciated. A warm thanks to the 'genotypes' of the canola breeding group Ray, An, Thabit, Hai, Siu Wah, Delbert, Kal, Vipan, Brent and Cindy. Also, many thanks to friends and colleagues (past and present) in the Department of Plant Science.

A very special thanks to Suzy, my best friend and companion, whose appearance into my life during the course of this work has made all this effort worthwhile.

Support from the Canadian Wheat Board, the Alberta Canola Producers Commission and Canola Research at the University of Alberta is gratefully acknowledged.

Table of Contents

CHAPTER I 1
- I.1. Introduction 1
  - I.1.a. Scope of the thesis 1
  - I.1.b. Canola production and nitrogen fertilization 1
  - I.1.c. Breeding nitrogen efficient *Brassica napus* genotypes 3
  - I.1.d. Genetic basis for breeding nitrogen efficient genotypes 3
- I.2. Research considerations for the implementation of the project 5
  - I.2.a. Nitrogen efficiency definitions 5
  - I.2.b. Plant parameters and growth stage 7
  - I.2.c. Recent research of nitrogen efficiency in *Brassica napus* 7
- I.3. Thesis objectives 8
- I.4. References 9

CHAPTER II 15

Effect of $NH_4^+:NO_3^-$ ratios on dry matter production and shoot growth components in rapeseed (*Brassica napus* L.). 15
- II.1. Introduction 15
- II.2. Materials and methods 17
  - II.2.a. Plant material and growing conditions 17
  - II.2.b. Plant growth measurements 17
  - II.2.c. Experimental design and statistical analysis 18
- II.3. Results 19
  - II.3.a. General visual symptoms and responses 19
  - II.3.b. Plant growth responses 19
  - II.3.c. Plant growth parameters 19
- II.4. Discussion 21
  - II.4.a. General visual symptoms and responses 21
  - II.4.b. Plant growth responses 21
  - II.4.c. Plant growth parameters 22
  - II.4.d. Change of preference for N forms 23
- II.5. Conclusions 23
- II.6. References 25

CHAPTER III 34

The use of intraspecific grafts to evaluate the influence of the root and shoot systems on nitrogen efficiency in rapeseed (*Brassica napus* L.) 34
- III.1. Introduction 34
- III.2. Materials and Methods 35
  - III.2.a. Plant culture and grafting technique 35
  - III.2.b. Measurement of plant growth and efficiency parameters 36
  - III.2.c. Experimental design and statistical analysis 36
- III.3. Results and Discussion 37
  - III.3.a. Grafting and graft treatment development 37
  - III.3.b. Comparisons between non-grafted controls: Pera versus Bronowski, GSL-I and Andor 38
  - III.3.c. Comparisons between non-grafted controls versus grafted controls 38
  - III.3.d. Comparisons of grafted controls: Pera versus Bronowski, GSL-I and Andor 40

III.3.e. Effects of the root system of the N-inefficient stock genotypes on the shoot system of the N-efficient stock genotype 40
III.3.f. Effects of the root system of the N-efficient genotype on the shoot system of the N-inefficient genotypes 42
III.4. Conclusions 43
III.5. References 44

CHAPTER IV 49
Time-course response of rapeseed (*Brassica napus* L.) to nitrogen supply: Selection of sampling time when screening for nitrogen efficient germplasm. 49
IV.1. Introduction 49
IV.2. Materials and Methods 51
IV.2.a. Plant material and growing conditions 51
IV.2.b. Plant growth and N efficiency parameters measured. 52
IV.2.c. Calculation of the points of inflexion of the curves 52
IV.2.d. Experimental design and statistical analysis. 53
IV.3. Results 54
IV.3.a. Visual growth responses to N supply 54
IV.3.b. Time-course responses of plant growth components to N supply 54
IV.3.c. Time-course responses of nitrogen efficiency 55
IV.3.d. Determining the transition of non-stress to stress N environment 56
IV.4. Discussion 58
IV.4.a. Differential response of leaf area relative to shoot dry weight 58
IV.4.b. Transition from N sufficiency to N deficiency 59
IV.5. Conclusions 60
IV.6. References. 61

CHAPTER V 70
The effect of nitrogen on growth and nitrogen efficiency components in rapeseed (*Brassica napus* L.) late in the rosette stage. 70
V.1. Introduction. 70
V.2. Materials and Methods. 72
V.2.a. Plant materials and growing conditions 72
V.2.b. Plant growth and N efficiency components 72
V.2.c. Experimental design and statistical analysis 74
V.3. Results 75
V.3.a. General responses to N supply 75
V.3.b. Response of plant growth components to N supply 75
V.3.c. Estimates of static nitrogen efficiency components 76
V.3.d. Estimates of response-rates N efficiency components 76
V.4. Discussion 78
V.5. Conclusions 79
V.6. References 80

CHAPTER VI 88
Variability of nitrogen efficiency components in rapeseed (*Brassica napus* L.) germplasm grown at deficient and sufficient nitrogen levels 88
VI.1. Introduction 88
VI.2. Materials and Methods 90
VI.2.a. Plant material and growing conditions 90
VI.2.b. Plant growth and N efficiency parameters 90

VI.2.c. Experimental design and statistical analysis 92
VI.3. Results and Discussion 93
VI.3.a. General observations 93
VI.3.b. Visual plant characteristics 93
VI.3.c. Genotypic variability of growth parameters 94
VI.3.d. Genotypic differences for static N efficiency components 95
VI.3.e. Genotypic differences for response-rate N efficiency components 96
VI.4. Conclusions 98
VI.5. References 99

CHAPTER VII 109
Genetic analysis of nitrogen efficiency components of doubled haploid progenies derived from a reciprocal cross of two rapeseed (*Brassica napus* L.) cultivars 109
VII.1. Introduction 109
VII.2. Materials and Methods 111
VII.2.a. Plant material and growing conditions 111
VII.2.b. Plant growth and N efficiency parameters 112
VII.2.c. Experimental design and statistical analysis 113
VII.3. Results 114
VII.3.a. General observations 114
VII.3.b. Responses of plant parameters 114
VII.3.c. Responses of the static N-efficiency parameters 115
VII.3.d. Responses of response-rate N-efficiency parameters 117
VII.4. Discussion 118
VII.4.a. Genotype × environment interactions 118
VII.4.b. Differences between DH populations (maternal effects) 119
VII.4.c. Efficiency components (genetics) 119
VII.4.d. Selection of N-efficiency parameters 120
VII.5. Conclusions 121
VII.6. References 122

CHAPTER VIII 134
Summary 134
VIII.1. General Discussion 134
VIII.2. Conclusions 136
VIII.3. References 136

APPENDIX I 137
List of rapeseed (*Brassica napus* L.) genotypes used for the screening of N-efficiency components in Chapter VI. 137

APPENDIX II 139
Original data obtained in both screenings (screening 1 and screening 2) of rapeseed (*Brassica napus* L.) genotypes for N efficiency described in Chapter IV. 139

APPENDIX III 146
Comparisons between transformed (ln) and non-transformed TOTN data selected from Chapter IV (Fig. IV.2a) 146

APPENDIX IV 147
Pairwise comparisons between genotypes used in the reciprocal crosses to develop DH lines (Chapter VII) 147

APPENDIX V 148

Responses of plant growth and nitrogen efficiency components of rapeseed (*Brassica napus* L.) when grown in a deficient (1.5 mmol) and a sufficient (5.1 mmol) nitrogen environment 148

List of Tables

Table I.1. Example of calculations illustrating N-efficiency parameters used in this thesis. Values used were taken from Fig. IV.2.a. and were graphed in Fig. I.1. (opposite page). 14

Table II.1. The effect of N-form ratio ($NH_4^+:NO_3^-$) on the relative growth rate of *Brassica napus* cv. Alto. 33

Table III.1. The effect of deficient (1.5 mmol) and sufficient (5.1 mmol) nitrogen supply on the expression of N-efficiency parameters of four *Brassica napus* (L.) genotypes. Partial data set obtained from Chapter 6. (Full results of both screenings were tabulated in Appendix 2) 46

Table III.2. Analysis of variance (ANOVA) for the expression of N-efficiency parameters of graft treatments of *Brassica napus* (L) 47

Table III.3. Comparisons of main and interaction estimates effects between graft treatments of *Brassica napus* (L). for shoot expression of N-efficiency parameters 48

Table IV.1. The effect of nitrogen supply on the number of days after seeding (DAS) that LA and TOTN curves underwent their point of inflexion (DAS_{PI}) and the DAS for the Knot point of NUE_{SDW}. 69

Table VI.1. Response of plant growth and static nitrogen efficiency parameters of two screenings of *Brassica napus* germplasm when grown in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. 101

Table VI.2. Response-rate nitrogen efficiency parameters of two screenings of *Brassica napus* germplasm in response to a 3.6 mmol N increment. 102

Table VI.3. Simple linear correlation coefficients of plant growth parameters of two screenings of *Brassica napus* germplasm when grown in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. 103

Table VI.4. Simple linear correlation coefficients of plant growth parameters with N efficiency parameters of two screenings of *Brassica napus* germplasm when grown in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. 104

Table VI.5. Simple linear correlation coefficients of N efficiency parameters of two screenings of *Brassica napus* germplasm when grown in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. 105

Table VI.6. Simple linear correlation coefficients of plant growth parameters with N efficiency parameters of two screenings of *Brassica napus* germplasm when grown in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. 106

Table VI.7. Simple linear correlation coefficients of static N efficiency parameters with response rate N efficiency parameters of two screenings of *Brassica napus* germplasm when grown in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. 107

Table VI.8. Simple linear correlation coefficients of response-rates N efficiency parameters of two screenings of *Brassica napus* germplasm in response to a 3.6 mmol N increment. 108

Table VII.1. Mean performance of plant components of DH progenies derived from Andor×Taiwan and Taiwan×Andor crosses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. 125

Table VII.2. Mean squares and errors of plant components associated with the DH progenies derived from Andor×Taiwan and Taiwan×Andor crosses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. Analysis was a randomized complete block. 126

Table VII.3. Comparisons of normality and equality of distribution of plant components of DH progenies derived from Andor×Taiwan and Taiwan×Andor croses and screened in a deficient (1.5 mmol) and sufficient (5.1 mmol) N environment. 127

Table VII.4. Mean performance of static N efficiency parameters of DH progenies derived from Andor×Taiwan and Taiwan×Andor crosses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. 128

Table VII.5. Mean squares and errors of static N efficiency parameters associated with the DH progenies derived from Ando×Taiwan and Taiwan×Andor crosses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. Analysis was a randomized complete block. 129

Table VII.6. Comparisons of normality and equality of distribution of static N efficiency components of DH progenies derived from Andor×Taiwan and Taiwan×Andor croses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. 130

Table VII.7. Mean performance of response-rate N efficiency parameters of DH progenies derived from Andor×Taiwan and Taiwan×Andor crosses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. 131

Table VII.8. Mean squares and errors of response-rate N efficiency parameters associated with the DH progenies derived from Andor×Taiwan and Taiwan×Andor crosses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. Analysis was a randomized complete block. 132

Table VII.9. Comparisons of normality and equality of distribution of response-rate N efficiency parameters of DH progenies derived from Andor×Taiwan and Taiwan×Andor croses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. 133

List of Figures

Fig. I.1. The effect of 1.5 and 8.0 mmol N on shoot dry weight of *Brassica napus* L. over 35 days after seeding. Data has been graphed to illustrate the different N-efficiency parameters used throughout the thesis. Refer to the text and Table I.1. (opposite page) for further explanations. 13

Fig. II.1. The effect of N-form ratio (% $NH_4^+:NO_3^-$) on dry weight accumulation of total shoots (a), leaves (b) and stems (c) of *Brassica napus* (L.) cv. Alto over time.................... 29

Fig. II.2. The effect of N-form ratio (% $NH_4^+:NO_3^-$) on leaf area (a) and leaf area ratio (b) of *Brassica napus* (L.) cv. Alto over time. 30

Fig. II.3. The effect of N-form ratio (% $NH_4^+:NO_3^-$) on leaf fraction (a) and specific leaf area (b) of *Brassica napus* (L.) cv. Alto over time.................... 31

Fig. II.4. The effect of N-form ratio (% $NH_4^+:NO_3^-$) on leaf density (a) of *Brassica napus* (L.) cv. Alto over time. 32

Fig. IV.1. The effect of N supply on the time course of shoot dry weight (a) and leaf area (b) of *Brassica napus* cv. Alto.................... 65

Fig. IV.2. The effect of N supply on the time course of total nitrogen uptake (a) and leaf area ratio (b) of *Brassica napus* cv. Alto.................... 66

Fig. IV.3. The effect of N supply on the time course of nitrogen use efficiency on a shoot dry weight basis (a) and leaf area basis (b) of *Brassica napus* cv. Alto.................... 67

Fig. IV.4. The effect of N supply on the time course of efficiency of utilization on a shoot dry weight basis (a) and leaf area basis (b) of *Brassica napus* cv. Alto.................... 68

Fig. V.1. The effect of N supply on shoot dry weight (SDW) and leaf area (LA) of *Brassica napus* cv. Alto plants harvested at 28 days after seeding.................... 83

Fig. V.2. The effect of increasing N supply on number of leaves (LNUM; a), leaf area ratio (LAR; b) and plant density (DENSITY; c) of *Brassica napus* cv. Alto plants harvested at 28 days after seeding.................... 84

Fig. V.3. The (a) effect N supply on total N uptake (TOTN) and the (b) response of shoot dry weight (SDW) and leaf area (LA) to total N uptake (TOTN) of B. napus cv. Alto plants harvested at 28 days after seeding.................... 85

Fig. V.4. The effect of N supply on (a) nitrogen use efficiency (NUE) and (b) on efficiency of utilization (EU), on a SDW and LA basis, of *Brassica napus* cv. Alto plants harvested at 28 days after seeding. 86

Fig. V.5. Estimates of (a) apparent recovery of N (AR) and (b) agronomic efficiency and (c) physiological efficiency on a shoot dry weight basis of *Brassica napus* cv. Alto plants harvested at 28 days after seeding.................. 87

List of Abbreviations

AE_{LA} = agronomic efficiency on a leaf area basis	(cm^2 mmol^{-1} N)
AE_{SDW} = agronomic efficiency on a shoot dry weight basis	(g mmol^{-1} N)
AR = apparent recovery	(%)
EU_{LA} = efficiency of utilization on a leaf area basis	(cm^4 mmol^{-1} N)
EU_{SDW} = efficiency of utilization on a shoot dry weight basis	(g^2 mmol^{-1} N)
LA = leaf area	(cm^2)
LAR = LA/SDW = leaf area ratio	(cm^2 g^{-1})
LD = LFW/LDW = leaf density	(%)
LDW = leaf dry weight	(g)
LF = LDW/SDW = leaf fraction	(%)
LFW = leaf fresh weight	(g)
LNUM = number of leaves per plant	
NUE_{LA} = nitrogen use efficiency on a leaf area basis	(cm mmol^{-1} N)
NUE_{SDW} = nitrogen use efficiency on a shoot dry weight basis	(g mmol^{-1} N)
PE_{LA} = physiological efficiency on a leaf area basis	(cm^2 mmol^{-1} N)
PE_{SDW} = physiological efficiency on a shoot dry weight basis	(g mmol^{-1} N)
SDW = LDW + STDW = total shoot dry weight	(g)
SFW = shoot fresh weight	(g)
SLA = LA/LDW = specific leaf area	(cm^2 g^{-1})
SLW = LDW/LA = specific leaf weight	(g cm^{-2})
STDW = stem dry weight	(g)
TOTN = total N absorbed	(mmol^{-1} N)

CHAPTER I

I.1. Introduction

I.1.a. Scope of the thesis

The topic of this thesis deals with the identification and breeding potential of nitrogen (N) efficient rapeseed (*Brassica napus* L.) germplasm. Among the essential plant nutrients, N has received the greatest attention since its discovery as an essential nutrient. Subsequently, a large volume of literature has been gathered and a number of books (eg. Baligar and Duncan, 1990), and monographs (eg. Graham, 1984) have been written on all aspects of N and its interaction in plants. It is not, however, the aim of this introduction to cover all aspects of N and its interactions, nor is the aim to cover nutrient or N efficiency in plants in its entirety. It is intended to provide an introduction and a context to the research presented in this thesis. In particular, it is intended to set the study of N efficiency in crop plants in a plant breeding context.

It is the purpose of this thesis to provide insight into the nature of N efficiency in rapeseed (*B. napus*) and to determine its possible use in a breeding program.

I.1.b. Canola production and nitrogen fertilization

Canola in Alberta accounts for approximately 34% of the total Canadian canola production in 1997 (Anonymous, 1997). Canola has higher nitrogen (N) requirements than either wheat or barley, and more than half of the requirement is removed with the seed crop (Anonymous, 1985). Furthermore, the requirement for N appears to be increasing because of cropping practices in Alberta (crop-crop-fallow or near continuous cropping) which have resulted in a 30 to 50% loss of soil organic matter and of the resulting plant nutrients, particularly N (Miller, 1983; Lickacz and Penny, 1985). Because of the short growing season, only about one quarter of the total N required for high yields of oilseed crops is supplied from N released from soil organic matter during the growing season (Anonymous, 1984). Higher yielding canola varieties will generally respond to higher rates of N application than those with lower yield potential (Anonymous, 1984). Thus, as new cultivars with higher yield potential are developed, N becomes one of the most commonly limiting nutrients. Maintaining high canola yields will require higher input rates of N fertilizer (Miller, 1983; Lickacz and Penny, 1985).

With the increasing rate of N fertilizer use N has become the major cause of soil acidification in Western Canada. Hoyt *et al.* (1981) projected that 25% of the soils in

Alberta would be acid by 1985, however, this projection has yet to be confirmed. Environmental concerns over N leaching which endangers ground water supplies has also increased in recent years. High rates of nitrogen fertilization are thought to be a major contributor to nitrate contamination in ground water in Europe and North America (Strebel *et al.*, 1989; Power and Shepers, 1989). New laws are emerging which restrict fertilizer use (N in particular) in certain regions so as to protect ground water. In Baden-Wurttemberg, Germany, a maximum nitrate limit has already been fixed for these areas (Schinkel and Mechelke, 1990). Water-quality problems related to agricultural development may well jeopardize the industry's ability to sustain itself in its current form.

Agriculturally related water-quality problems do not yet appear to be a problem in Alberta, even in irrigated areas which represent the most intensive, large-scale agricultural development on the Prairies (Paterson, 1991). According to Paterson (1991) however, there is insufficient experimental data on which to fully base this conclusion. Nevertheless, nitrate contamination of groundwater and surface water is becoming an issue of increasing concern in Alberta (Paterson, 1991). The main problem relates to nitrate leaching into the groundwater (Paterson, 1991). Effective methods of reduction of N-fertilizer usage are warranted.

Environmental and economic problems have provided a platform for a renewed emphasis on alternative cropping practices. Regardless of the impact that the "green revolution" may have had on agriculture, much needs to be done to improve the cropping practices in developing countries. Agricultural systems such as sustainable agriculture, low input agriculture and organic farming have taken centre stage as agricultural models for the next century.

A major focus of attention in many proposed agricultural models has been the improvement of fertilizer use by crop plants. Soil fertility problems have always focused on the adjustment of the soil to fit the crop. Although technology is available to detect and correct nutrient deficiencies where they exist, economics become a significant factor with rising costs of fertilizers and amendments. An economical approach to soil fertility problems emphasizes tailoring the crop to fit the soil. Selection for genotypic variation in N uptake and utilization by plants could be an effective avenue for crop improvement (eg. Gerloff, 1963; Vose, 1963; Gabelman, 1976; Clark and Duncan, 1991). Breeding of canola cultivars with improved efficiency in N use, whether through an increase in yield at static levels of N or through the maintenance of current yield with a reduction in N inputs, would benefit the canola grower and reduce the negative impact on the environment.

Producer profit margins have eroded substantially in recent years as the cost of inputs (particularly fertilizers) have increased while crop values have been declining. Greater N-fertilizer-use efficiency could contribute to a reversal of this trend. The possibility of exploiting genotypic differences in absorption and utilization of N to improve efficiency of N fertilizer-use, or of obtaining higher productivity of canola on N-deficient soils would greatly benefit canola producers wishing to maximize efficiency of resource utilization. Furthermore, reduction of N-fertilizer-use would reduce acidifying effects and the danger of ground water contamination due to N-NO_3^- leaching. Plant breeders should be preparing for this reduction because it takes many years to develop new, adapted cultivars.

I.1.c. Breeding nitrogen efficient *Brassica napus* genotypes

Several reviews of genotypic variation in nutrient uptake and utilization by plants have concluded that selection for such characters could be an effective avenue for crop improvement (eg. Gerloff, 1963; Vose, 1963; Gabelman, 1976; Clark and Duncan, 1991). Given: (a) the current high costs of nitrogenous fertilizers, (b) the increasing concern about water contamination due to leaching of fertilizer nitrogen (N), (c) the strong soil acidifying action of NH_4-based fertilizers (eg. Hoyt *et al.*, 1981) and (d) the existence of genotypic variation in N uptake and N utilization in crop species (eg. Clark, 1990), attempts to improve the agronomic performance of crop species through selection for more efficient use of N now seem to be justified.

A breeding program involving selection for 'N uptake efficiency' and 'N utilization efficiency' can be divided into two major objectives (Yau and Thurling, 1987; Vose, 1990). First, the breeder may wish to improve yield in a system in which static levels of N fertilization are maintained. Alternatively, the breeding program could be designed to produce cultivars capable of yielding at current levels under significantly reduced inputs of N. Improved efficiency in N usage by crops, whether through an increase in yield at static levels of N or through the maintenance of current yield with a reduction in N inputs, would benefit the canola grower.

I.1.d. Genetic basis for breeding nitrogen efficient genotypes

Genetic variability and heritability for important enzyme systems related to the efficient use of N by plant crops such as N uptake rate, metabolism, photosynthesis, N fixation and dark respiration have been reported for numerous plant species (eg. Hageman et al., 1967; Hobbs and Mahon, 1982; Sherrard et al., 1984; Vose and Breese, 1964; Wilson, 1981; Clark, 1990). In these cases, adequate, but not ideal, methods for measurement of traits were developed. Unfortunately, under field conditions the associations between the various physiological or biochemical traits and yields have not been consistent or strong enough to lead to widespread commercial utilization (Sherrard et al., 1985).

Little detailed information on the genetic control of N uptake and N utilization is available for any crop. In the case of N uptake, broad-sense heritabilities in wheat at anthesis and maturity were 0.36 and 0.42 respectively (Austin et al., 1977). Narrow-sense heritability estimates ranging from 0.19 to 0.45 were obtained from analyses of variation in two perennial ryegrass cultivars, for total N-uptake (Holmes, 1965). Low heritabilities for nitrogen uptake were obtained in both solution culture (Holmes, 1967) and field (Rogers and Thomson, 1970) studies with *Lolium perenne*. However, these low estimates contrasted with a heritability of 0.73 for N-uptake reported in a later study with *Lolium perenne* and *L. multiflorum* (Goodman, 1977). Genetic studies of N utilization in perennial ryegrass (Thomson and Rogers, 1970) and tomato (O'Sullivan et al., 1974) indicated that non-additive gene effects were most important and the narrow-sense heritability was low.

In the study of N uptake and N utilization of plants, graminaceous species have received most of the attention in the literature (Macduff and Wild, 1989). Among dicot species, the emphasis has been on N fixing species. In the case of the non-leguminous oilseed rape (*Brassica napus* L.) crop research on N has been sporadic and mainly on the agronomic aspects of N response. Uptake of N by oilseed rape is usually large (Lefevre and Lefevre, 1957), with the highest levels reported at the rosette stage (stage 2; Harper, 1973). Intensive N uptake occurs between germination and the beginning of flowering, after which N uptake declines (Rood et al., 1984). *Brassica napus* has the capacity to respond to applied N above 150 kg N per hectare (Holmes, 1980). In vegetative growth stages, the mobility of internal N compounds determines yield components (Krogman and Hobbs, 1975) such as number of axillary branches or number of flowers. During the early stages of leaf (Freyman et al., 1973; Clarke, 1978) and pod development, N is predominantly present as protein. However, during the course of senescence, N is exported from vegetative parts to the seed (Kullmman and Geisler, 1986). According to Allen and Morgan (1972) N level regulates the number of pods per plant and the number of seeds per pod during the period of pod development. Furthermore, it is likely that the effect of N is achieved indirectly through an increase in the supply of assimilates to the flowers and young pods (Allen and Morgan, 1972). It has been reported that the N supply of young hulls and seeds is provided primarily by N translocation within plants (Pate, 1971 as indicated by Kulmman and Geisler, 1986). *Brassica napus* was found to absorb similar amounts of total N, irrespective of whether it was supplied as NH_4^+, NO_3^-, or $NH_4^+ + NO_3^-$, the discrimination occurring at root temperatures between 3-25 °C (Macduff et al., 1987a,b).

Evidence for genotypic variation in N uptake and N utilization in *B. napus* has been reported (Yau and Thurling, 1986; Gerath and Schweiger, 1991; Gerath and Balko, 1995). The low heritabilities (0.0 to 0.45) for N uptake and N utilization of *B. napus* obtained by Yau and Thurling (1987) were consistent with most of the few recorded estimates in other plant species. The very low heritabilities for N utilization in *Lolium perenne* and *B. napus* may reflect the absence of significant genotypic variation under a non-limiting N supply. *Brassica napus* cultivars grown at different N levels (Yau and Thurling, 1986) were shown to have significant differences in N utilization only at the lowest N level, which was clearly limiting plant growth. Thus, Yau and Thurling (1986) concluded that there would be little scope for selecting genotypes capable of yielding at current levels under reduced nitrogen inputs. However N-uptake and utilization measurements may be of some value in selection for higher yield at current nitrogen application rates if used as an index in conjunction with other selection parameters in a breeding program (Yau and Thurling, 1986).

I.2. Research considerations for the implementation of the project

I.2.a. Nitrogen efficiency definitions

Adequate but not ideal methods for measurement of N-efficiency have been developed. In general, 'N efficiency' research has taken a 'from the bottom up' approach. That is, emphasis has been to select for a specific biochemical, physiological or structural trait which has been correlated to N efficiency. Thus far the results obtained have not been commercially useful. Most studies of plant stress have also focused on responses of a single process to a single stress factor over a short time. Yet as a stress is imposed, traits usually exhibit a cascade of responses occurring on different time scales and involving biochemical and morphological adjustments. Since plants are integrated units, stress responses cannot be fully evaluated except in the context of the whole plant. The approach to be taken in this research will be from the 'top down' and in the context of the whole plant.

Several definitions and terms appear in the literature relative to nutrient efficiency (Clark, 1990). For the purpose of this research six definitions for nutrient efficiency will be used:

Nitrogen use efficiency (NUE) = SDW/TOTN (g $\mathrm{mmol^{-1}}$ N)

From Siddiqi and Glass (1981),

Efficiency of utilization (EU) = SDW (SDW/TOTN) ($\mathrm{g^{-2}\ mmol^{-1}}$ N)

From Craswell and Godwin (1984),

Physiological efficiency (PE) = $(\mathrm{SDW_{High\ N}} - \mathrm{SDW_{Low\ N}})/(\mathrm{TOTN_{High\ N}} - \mathrm{TOTN_{Low\ N}})$ (g $\mathrm{mmol^{-1}}$ N)

Agronomic efficiency (AE) = $(\mathrm{SDW_{High\ N}} - \mathrm{SDW_{Low\ N}})$/net N applied) (g $\mathrm{mmol^{-1}}$ N)

Apparent recovery (AR) = $((\mathrm{TOTN_{High\ N}} - \mathrm{TOTN_{Low\ N}})$/net N applied) 100 (%)

Where: SDW = shoot dry weight, TOTN = total nitrogen absorbed $((((\mathrm{SDW} \times \mathrm{N\%})/100)/14.0) \times 1000)$ (mmol). Low N = N-treatment taken as control N-treatment; High N = N-treatment higher than control; net N applied = difference between High N- and Low N-treatments. By replacing the SDW term with LA in the equations, efficiency components on a LA basis were obtained. The relationship among the N-efficiency parameters used in this study have been shown in Fig. I.1. and Table I.1.

As explained by Craswell and Godwin (1984), apparent recovery depends upon the assumption that fertilized and control plants absorb the same total amount of soil N; hence the term "apparent". Apparent N recovery reflects the efficiency of the crop in obtaining fertilizer N from the soil. Physiological efficiency can be viewed as the efficiency with which plants utilize N in the plant for the synthesis of shoot yield. Agronomic efficiency is the product of the physiological efficiency and the apparent recovery and thus reflects the overall efficiency with which applied N is used. As outlined by Novoa and Loomis (1981), increases in either or both the apparent N recovery and the physiological efficiency will increase the agronomic efficiency.

I.2.b. Plant parameters and growth stage

In a plant breeding program where several hundred genotypes are being evaluated for a range of characteristics on a regular basis, the earlier in the plant growth cycle the evaluation is performed, the more efficient the system becomes. When screening for N efficient *B. napus* germplasm, all factors involved in the protocol need to be evaluated. The developmental stage, plant growth and N efficiency components used in screening N efficient *B. napus* first need to be defined (Clark, 1990) and then related to the final economic yield of the crop (Duncan and Baligar, 1990). Selection of the growth stage and plant components is complicated by the fact that the sensitivity to nutritional deficiencies and toxicities may change as the plant goes through various growth and developmental stages (Duncan and Baligar, 1990). In the case of *B. napus*, the rosette stage is an important growth stage which has a large influence on the overall final yield (see Thurling, 1993 and references therein). The rosette stage begins when the first normal leaf is unfolded, and terminates when the stem begins to elongate (Harper, 1973). This relationship with final yield enables the growth components of the rosette stage to become potential diagnostic markers for selecting N efficient *B. napus* germplasm. Thus, the rosette stage was the growth stage at which most of the assays were done throughout the research reported here.

I.2.c. Recent research on nitrogen efficiency in *Brassica napus*

Thus far no germplasm specifically efficient in N uptake and utilization has been developed and released for commercial use. In the case of rapeseed, only two research projects have been undertaken. In Australia, Thurling's research program on N-efficiency lasted for only three years (Dr. N. Thurling, personal communication). In Germany, Gerath and Schweiger (1991) and Gerath and Balko (1995) are still continuing their research program, but it has been impossible to obtain detailed information of their progress or seed samples for research purposes. In Canada, there are no ongoing research projects in N efficiency of *B. napus*. Thus, to my knowledge, the group in Germany and our research group are the only ones researching N-efficiency of *B. napus*. Recently, a new multidiciplinary initiative, ranging from molecular to whole plant approaches, has been initiated in Germany to improve the N utilization of rapeseed (Dr. W. Horst, personal communication).

I.3. Thesis objectives

The main objectives of this thesis are as follows: (1) to determine whether there is genetic variation for 'N efficiency' amongst germplasm accessions of *B. napus*, (2) to determine if the genetic variation for 'N efficiency' is potentially useful in a breeding program, and (3) to obtain information on the genetic nature of N uptake and utilization efficiency in *B. napus*.

A number of studies were carried out to develop a screening technique for N efficient *B. napus* germplasm. In particular, the effect of $NH_4^+:NO_3^-$ ratios on dry matter production and shoot growth components in rapeseed (Chapter II) as well as the use of intraspecific grafts to evaluate the influence of the root and shoot systems on N efficiency in rapeseed plants were studied (Chapter III). A time-course response to N supply was performed to select an appropriate sampling time for screening N efficient germplasm (Chapter IV). Also, the effects of N supply on rapeseed growth and N efficiency components late in the rosette stage were determined (Chapter V). These studies provided the basis for a screening technique as well as providing information regarding the N efficiency components in rapeseed. The genotypic variability of N efficiency components of a large number of rapeseed genotypes was determined under deficient and sufficient N levels (Chapter VI). This was followed by a genetic analysis of N efficiency components of doubled haploid progeny derived from a reciprocal cross of rapeseed cultivars (Chapter VII). The thesis concludes with a summary chapter in which the results and conclusions of the previous chapters are brought together and a short discussion regarding the future prospects for breeding N efficient rapeseed germplasm is presented (Chapter VIII).

I.4. References

Allen E J and Morgan D G 1972 A quantitative analysis of the effects of nitrogen on the growth, development and yield of oilseed rape. J. Agric. Sci. Camb. 78, 315-324.

Anonymous 1997 Statistics: Acrages and yields. Canola Council of Canada. Winnipeg, Canada.

Anonymous 1985 Canola Production in Alberta. Alberta Agriculture, Field Crops Branch. Agdex149/20-1.

Anonymous 1984 Alberta Fertilizer Guide. Agrifax. Alberta Agriculture. Agdex541-1.

Austin R B, Ford M A, Edrich J A and Blackwell R D 1977 The nitrogen economy of winter wheat. J. Agric. Sci., Camb. 88, 159-167.

Baligar V C and Duncan R R 1990 Crops as enhancers of nutrient use. Academic Press, Inc., San Diego, USA. 574 p.

Clarke J M 1978 The effects of leaf removal on yield and yield components of *Brassica napus*. Can. J. Plant Sci. 58, 1103-1105.

Clark R B 1990 Physiology of cereals for mineral nutrient uptake, use and efficiency. *In* Crops as enhancers of nutrient use. Eds. V C Baligar and R R Duncan. pp 131-209. Academic Press, Inc., San Diego, USA.

Clark R B and Duncan R R 1991 Improvement of plant mineral nutrition through breeding. Field Crops Res. 27, 219-240.

Craswell E T and Woodwin D C 1984 The efficiency of nitrogen fertilizers applied to cereals in different climates. Adv. Plant Nutr. 1, 1-55.

Duncan R R and Baligar V C 1990 Genetics, breeding, and physiological mechanisms of nutrient uptake and use efficiency: An overview. *In* Crops as enhancers of nutrient use. Eds. B C Baligar and R R Duncan. pp 3-35. Academic Press, San Diego, USA.

Freyman S, Charnetski W A and Crookston R K 1973 Role of leaves in the formation of seed in rape. Can. J. Plant Sci. 53, 693-694.

Gabelman W H 1976 Genetic potentials in nitrogen, phosphorus and potassium efficiency. *In* Plant adaptation to mineral stress in problem soils. Ed. M J Wright. pp 205-212. Cornell University Press, New York, USA.

Gerath H H and Balko C 1995 The influence of genotype and donor plant on the nitrogen efficiency of DH-lines of winter rape. *In* Proceedings GCIRC 9th international rapeseed congress: Rapeseed today and tomorrow. Cambridge, UK. Vol. 3, 813-815.

Gerath H H and Schweiger W 1991 Improvement of the use of nutrients in winter rape. A strategy of economically and ecologically responsible fertilizing. *In* Proceedings GCIRC eight international rapeseed congress. Saskatoon, Canada. Vol 4, 1197-1201.

Gerloff G C 1963 Comparative mineral nutrition in plants. Ann. Rev. Plant Physiol. 14, 699-704.

Goodman P J 1977 Selection for nitrogen responses in *Lulium* Ann. Bot. 41, 243-256.

Graham R D 1984 Breeding for nutritional characteristics in cereals. Adv. Plant Nutri. 1, 57-102.

Hageman R H, Leng E R and Dudley J W 1967 A biochemical approach to corn breeding. Adv. Agron. 19, 45-86.

Harper F R 1973 A key to standardize the description of growth stages in turnip rape, *Brassica campestris*. Can. Plant Dis. Surv. 53, 93-95.

Hobbs S L A and Mahon J D 1982 Variation, heritability and relationship to yield o physiological characters in peas. Crop Sci. 22, 773-779.

Holmes D P 1965 Nitrogen uptake and distribution. Rep. Welsh Pl. Breed. Stn. for 1964, 28-29.

Holmes D P 1967 Genetic control of nitrogen uptake. Rep. Welsh Pl. Breed. Stn. for 1966, 30-31.

Holmes M R J 1980 Nutrition of the oilseed rape crop. Applied Science Publishers Ltd., London, UK. 158 p.

Hoyt P B, Nyborg M and Ukrainetz H 1981 Degradation by acidification. *In* Agricultural Land, Our Disappearing Heritage - A Symposium. Proc. 18th Ann. Alta. Soil Sci. Workshop. Feb., 1981. pp 41-71. Alberta Soil & Feed Testing Laboratory, Alberta, Canada.

Krogman K K and Hobbs E H 1975 Yield and morphological response of rape (*Brassica campestris* L. cv. Span) to irrigation and fertilizer treatments. Can. J. Plant Sci. 55, 903-909.

Kullman A and Geisler G 1986 Translocation of labelled nitrogen in oilseed rape. *In* Fundamental, ecological and agricultural aspects of nitrogen metabolism in higher plants. Eds. H Lambers, J J Neeteson and I Stulen. pp 127-129. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

Lefevre G and Lefevre P 1957 Observations on the uptake of nutrients by winter rape. Agronomiq. Series A 8, 125-144.

Lickacz J and Penny D 1985 Soil Organic Matter. Agrifax. Agdex 536-1. Alberta Agriculture, Canada.

Macduff J H, Hopper M J and Wild A 1987a The effect of root temperature on growth and uptake of ammonium and nitrate by *Brassica napus* L. cv. Bienvenu in flowing solution culture. II. Uptake from solutions containing NH_4NO_3. J. Exp. Bot. 38, 53-66.

Macduff J H, Hopper M J, Wild A and Trim F E 1987b Comparison of the effects of root temperature on nitrate and ammonium nutrition of oilseed rape (*Brassica napus* L.) in flowing solution culture. I. Growth and uptake of nitrogen. J. Exp. Bot. 38, 1104-1120.

Macduff J H and Wild A 1989 Interactions between root temperature and nitrogen deficiency influence preferential uptake of NH_4^+ and NO_3^- by oilseed rape. J. Exp. Bot. 40, 195-206.

Miller M H 1983 Soil limitations to crop productivity in Canada. Can. J. Plant Sci. 63, 23-32.

Novoa R and R S Loomis 1981 Nitrogen and plant production. Plant Soil 58, 177-204.

O'Sullivan J, Gabelman W H and Gerloff G C 1974 Variation in efficiency of nitrogen utilization in tomatoes (*Lycopersicon esculentum* Mill.) grown under nitrogen stress. Am. Soc. Hort. Sci. 99, 543-547.

Paterson B A 1991 Effects of irrigation and drainage on surface and groundwater quality. Proc. 28th Ann. Alta. Soil Sci. Workshop. Feb., 1981. pp 31-45. Alberta Soil & Feed Testing Laboratory, Alberta, Canada.

Power J F and Shepers J S 1989 Nitrate contamination of groundwater in North America. *In* Agriculture, Ecosystems and Environment. Eds. H Bouwer and R S Bowman. pp 95-108. Elsevier Science Publishers, B. V., Amsterdam, The Netherlands.

Rogers H H and Thomson A J 1970 Aspects of the agronomy and genetics of quality components in a diallel set of progenies of *Lolium perenne*. L. J. Agric. Sci., Camb. 75, 145-158

Rood S B, Major D J, Carefoot J M and Bole J B 1984. Seasonal distribution of nitrogen in oilseed rape. Field Crops Res. 8, 333-340.

Schinkel B and Mechelke W 1990 A method to estimate the prospect of specific breeding for nutrient efficiency. *In* Genetic aspects of plant mineral nutrition. Eds. N El Bassam, M. Dambroth and B. C. Loughman. pp 449-456. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Sherrard J H, Lambert R J, Bellow F E, Dunand R T, Messner M J, Wilson M R, Winkels C S and Hageman R H 1985 Use of physiological traits, especially those of nitrogen metabolism for selection of maize. *In* Biochemical Basis of Plant Breeding Vol II, Nitrogen Metabolism. Ed. C A Neyra. pp 109-130. CRC Press, Inc., Boca Raton, USA.

Sherrard J H, Lambert R J, Messner M J, Bellow F E and Hageman R H 1984 Plant breeding for efficient use of nitrogen. *In* Nitrogen in Crop Production. Ed. R D Hauck. pp 363-378. ASA, Madison, USA.

Siddiqi M Y and Glass A D M 1981 Utilization index: a modified approach to the estimation and comparison of nutrient utilization efficiency in plants. J. Plant Nutr. 4, 289-302.

Strebel O, Duynisveld W H M and Bottcher J 1989 Nitrate pollution of groundwater in Western Europe. Agric. Ecosystems Environ. 26, 189-214.

Thomson A J and Rogers H H 1970 Recovery and utilization of applied nitrogen by a diallel set of progenies of *Lolium perenne* L. J. Agric. Sci., Camb. 75, 159-167.

Thurling N 1993 Physiological constraints and their genetic manipulation. *In* Breeding oilseed brassicas. Eds. K S Labana, S S Banga and K S Banga pp 44-66. Springer-Verlag, Berlin, Germany.

Vose P B 1963 Varietal differences in plant nutrition. Herbage Abst. 33, 1-13.

Vose P B 1990 Screening techniques for plant nutrient efficiency: Philosophy and methods. *In* Genetic aspects of plant mineral nutrition. Eds. N El Bassam, M Dambroth and B C Loughman. pp 283-289. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Vose P B and Breese E L 1964 Genetic variation in the utilization of nitrogen by ryegrass species *Lolium perenne* and *L. multiflorum*. Ann. Bot. 28, 251-270.

Wilson D 1981 Breeding for morphological and physiological traits. *In* Plant Breeding II. Ed. K J Frey. pp 233-290. Iowa State University Press, Ames, USA.

Yau S K and Thurling N 1986 Variation in nitrogen response among spring rape (*Brassica napus*) cultivars and its relationship to nitrogen uptake and utilization. Field Crops Res. 16, 139-155.

Yau S K and Thurling N 1987 Genetic variation in nitrogen uptake and utilization in spring rape (*Brassica napus* L.) and its exploitation through selection. Plant Breeding 98, 330-338.

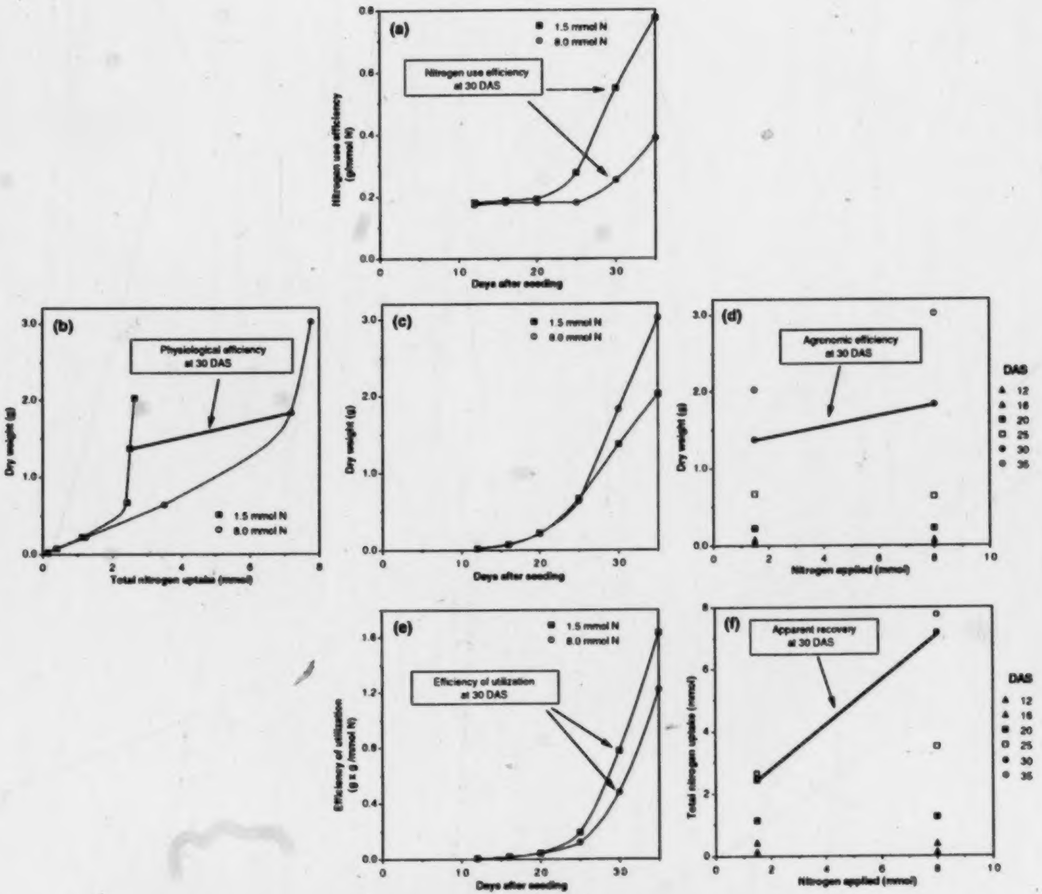


Fig. I.1. The effect of 1.5 and 8.0 mmol N on shoot dry weight of *Brassica napus* L. over a 35 days period after seeding. Data has been graphed to ilustrates the different N-efficiency parameters used throughout the thesis. Refer to the text and Table I.1. (opposite page) for further explanations.

Static N-efficiency parameters

Fig. I.1.a. Nitrogen use efficiency

$$NUE_{1.5} = \frac{1.38}{2.51} = 0.55 \text{ g mmol}^{-1} \text{ N}$$

$$NUE_{8.0} = \frac{1.84}{7.18} = 0.26 \text{ g mmol}^{-1} \text{ N}$$

Fig. I.1.e. Efficiency of utilization

$$EU_{1.5} = 1.38 \times \frac{1.38}{2.51} = 0.76 \text{ g}^2 \text{ mmol}^{-1} \text{ N}$$

$$EU_{8.0} = 1.84 \times \frac{1.84}{7.18} = 0.47 \text{ g}^2 \text{ mmol}^{-1} \text{ N}$$

Response-rate N-efficiency parameters

Fig. I.1.b. Physiological efficiency

$$PE = \frac{1.84 - 1.38}{7.18 - 2.51} = 0.10 \text{ g mmol}^{-1} \text{ N}$$

Fig. I.1.f. Apparent recovery

$$AR = \frac{7.18 - 2.51}{6.5} \times 100 = 0.72 \ \%$$

Fig. I.1.d. Agronomic efficiency

$$AE = \frac{1.84 - 1.38}{6.5} = 0.07 \text{ g mmol}^{-1} \text{ N}$$

Table I.1. Example of calculations illustrating N-efficiency parameters used in this thesis. Values used were taken from Fig. IV.2.a. and were graphed in Fig. I.1. (opposite page).

CHAPTER II

Effect of $NH_4^+:NO_3^-$ ratio on dry matter production and shoot growth components in rapeseed (*Brassica napus* L.).

II.1. Introduction

Nitrate (NO_3^-) and ammonium (NH_4^+) are the two major sources of nitrogen (N) available for plant uptake (Marschener, 1995). Numerous studies have shown that NH_4^+, as the sole source of N, is deleterious to the growth of several crop species including triticale, wheat, rye (Gashaw and Mugwira, 1981), tomato (Magalhaes and Wilcox, 1983), radish (Goyal et al., 1982), sunflower (Kaiser and Lewis, 1991), millet (Smith et al., 1990), sugar beet (Krstic et al., 1986) and flatpea (Shen et al., 1990). A notable exception is rice, which is more tolerant to high NH_4^+ concentration and thus uses NH_4^+ preferentially at an early growth stage (Ismunadji and Dijkshoorn, 1971). However, additions of small amounts of NH_4^+ to NO_3^- fertilizers have been reported to increase growth of many plant species including triticale, rye (Gashaw and Mugwira, 1981), wheat (Cox and Reisenauer, 1973), ryegrass (Reisenauer et al., 1982), corn (Warnecke and Barber, 1973), and sunflower (Kaiser and Lewis, 1991). These studies support the concept that most crop species grow optimally when supplied with both forms of N (Haynes and Goh, 1978). However, the relative effect on plant growth of the combined N-forms is influenced by a number of factors, including plant species (Gashaw and Mugwira, 1981), cultivar within a species (Krstic et al., 1986), and environmental growing conditions such as root zone temperature (Macduff and Wild, 1989; Macduff and Trim, 1986; Clarkson et al., 1992), pH of the growing medium (Fried et al., 1965; Jungk, 1970), concentration of the N supplied (Xu et al., 1992) and $NH_4^+:NO_3^-$ ratios (Errebhi and Wilcox, 1990; Smith et al., 1990). Furthermore, the favored N-form taken up by plants frequently changes with ontogeny (McKee, 1962; Dibb and Welch, 1976; Smith et al., 1990).

To select N-efficient rapeseed (*Brassica napus* L.) genotypes a screening protocol where N-treatments are standardized for maximum plant growth is required. In view of the wide range of plant responses to N-forms, information regarding the growth response of *B. napus* to varying $NH_4^+:NO_3^-$ ratio needs to be determined before proceeding with an N-efficiency screening methodology. Although information on the effect of N levels on *B. napus* is extensive (Holmes, 1980), information on the effects of N-form ratios is lacking. Thus, the objective of this study was to investigate the effect of $NH_4^+:NO_3^-$ ratio on growth of *B. napus* at an early growth stage. This information should provide a basis for selecting optimum N-form ratios when selecting N-efficient *B. napus* genotypes.

II.2. Materials and methods

II.2.a. Plant material and growing conditions

Plants of rapeseed (*Brassica napus* L.) cv. Alto were grown in 15 cm diameter plastic pots filled with a soil-free medium (Vermiculite + Peat Moss + Sand; 3:3:1 volume basis) under greenhouse conditions. The growing medium contained an average of 8.07×10^{-2} mmol total N g^{-1}. Sealed plastic bags were inserted into each pot to prevent nutrient loss through drainage holes. Two to six seeds were planted per pot, and thinned to one seedling seven days after seeding (DAS). A modified basal nutrient solution (Taylor and Foy, 1985) was added to each individual pot providing (μmol): K, 160; PO_4, 40; SO_4, 40; Mg, 15; Cl, 33; Mn, 0.2; B, 0.6; Zn, 0.05; Cu, 0.015; Na, 2.0; Mo, 0.01 EDTA, 1.0; Fe, 1.0. Iron was supplied as Fe-EDTA prepared from equimolar amounts of $FeCl_3$ and Na_2EDTA. Stock solutions of NH_4NO_3, $(NH_4)_2SO_4$, $Ca(NO_3)_2 \cdot 4H_2O$ and $CaSO_4 \cdot 2H_2O$ were combined to develop seven N-treatments consisting of the following $NH_4^+:NO_3^-$ ratios (%:%): 100:0, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100. Treatments provided a constant Ca level of 0.6 mmol and a variable S level ranging from 0.35 to 0.60 mmol. Commencing at 10 DAS, the basal nutrient solution (100 ml), together with 50 μmol N for each N-treatment, were added to each pot daily. At 16 DAS, N-treatment levels were increased to 100 μmol N. When necessary, plants were supplemented with distilled water and care was taken to prevent waterlogging.

Plants were grown at an ambient temperature of 20-25 °C and a relative humidity of 60-90%. Natural light was supplemented with 400 Watt, high pressure sodium lamps (Sylvania) attached to high intensity discharge fixtures, providing a mean basal photosynthetic photon flux density (PPFD) of 350 ± 20 μmol m^{-2} s^{-1} at soil surface height for 16 h daily.

II.2.b. Plant growth measurements

Shoots were harvested individually by cutting at the surface of the growth medium, at 14, 19, 24, 29 and 34 DAS. Plant measurements included leaf area (LA, cm^2), leaf fresh weight (LFW, g), leaf dry weight (LDW, g), stem dry weight (STDW, g) and total shoot dry weight (SDW = LDW + STDW, g). Leaf area was measured on a LI-COR LI3100 Area Meter (LI-COR Inc., Lincoln, Nebraska, USA). Samples were dried to constant weight at 65°C and weighed. Plant growth parameters were calculated according to Gardner et al. (1985) and consisted of leaf area ratio (LAR = LA/SDW, cm^2

g^{-1}), specific leaf area (SLA = LA/LDW, cm^2 g^{-1}), leaf density (LD = LFW/LDW, %), leaf fraction (LF = LDW/SDW, %) and specific leaf weight (SLW = LDW/LA, g cm^{-2}).

II.2.c. Experimental design and statistical analysis

The experimental design was a randomized complete block, consisting of seven N-treatments, five harvests, two plants per N-treatment and six replications. Homogeneity of variance was determined by Levene's test (Levene, 1960). Relative growth rate (RGR) of SDW accumulation over time was determined on transformed (ln) data (Gardner et al., 1985). Significance between means were determined by the least significance difference (LSD) or Duncan multiple range (DMR) tests (Gomez and Gomez, 1984) and estimated with the statistical software SAS/STATS (SAS Institute, 1992). Unless otherwise indicated, all levels of significance were at $p \leq 0.05$.

II.3. Results

II.3.a. General visual symptoms and responses

No N deficiency symptoms were observed during the experiment suggesting that N-treatments were sufficient for normal plant growth. Initiation of stem elongation was observed at about 28 DAS while, floral initiation was observed at about 34 DAS in all N-treatments. This indicated that increased NH_4^+% in the N-treatments did not alter ontogeny of *B. napus*.

II.3.b. Plant growth responses

The LDW and STDW increased significantly in N-treatments containing some NH_4^+ relative to N-treatments without NH_4^+ (Fig. II.1). This resulted in an overall increase in the total above-ground dry matter accumulation (SDW; Fig. II.1a). The total dry weight accumulation (SDW) did not appear to have leveled off at the end of the growth period (Fig. II.1a). Leaf area expansion, on the other hand, showed a leveling off above 28 DAS (Fig. II.2a). The leveling off in LA expansion (Fig. II.2a) was not accompanied by a concomitant leveling off of LDW (Fig. II.1b) but, it coincided with the transition from rosette to stem elongation growth stage. Although, at the end of the growth assay maximum LA expansion was produced by plants grown in 40% NH_4^+ (Fig. II.2a), it was only significantly different with the LA produced by plants grown in 10% NH_4^+. It did not appear that an optimum NH_4^+% level for maximum growth of SDW and LA existed.

II.3.c. Plant growth parameters

According to Levene's test the variance associated with SDW over the growth period was heterogeneous (analysis not included; Levene, 1960). Thus, relative growth rate of SDW over the growth period was estimated on transformed (ln) data. Significant differences for RGR among N-treatments were observed (Table II.1). A significant reduction of the RGR was obtained without any NH_4^+ in the N-treatment relative to plants grown with any amount of NH_4^+ in the N-treatments (Table II.1). Furthermore, there was not any significant difference amongst RGR of plants grown in N treatments with any amount of NH_4^+ in the medium.

Leaf area ratio reflects the "leafiness" of a plant (Gardner et al., 1985; Causton and Venus, 1981). Commencing at about 18 DAS, LAR declined steadily throughout the growth period for all N-treatments (Fig. II.2b). This coincided with a steady shift from rosette to stem elongation growth stage. The highest LAR at the end of the growth period was obtained without NH_4^+ in the medium, however, this was only significantly different with LAR level of plants grown with 20% NH_4^+ in the medium (Fig. II.2b).

The LF declined steadily over the growth period however, there were no significant differences between N-treatments at any harvest time (Fig. II.3a). The reduction in LF coincided with a shift towards a larger investment in stem formation, particularly at and above 28 DAS; during the stem elongation growth stage. In this case no changes in ranking of N-treatment effects for maximum LF over the growth period were observed.

The SLA declined over the growing period after 18 DAS for all N-treatments (Fig. II.3b). Unlike LF, the shape response curves of SLA (Fig. II.3b) were similar to the shape response curves of LAR (Fig. 1b). This indicated a major influence of SLA on LAR. The highest SLA towards the end of the growth period was obtained without NH_4^+ in the N-treatments, however, it was only significantly different with SLA of plants grown in 100% NH_4^+ in the medium (Fig. II.3b).

The LD commenced a decline at about 18 DAS across all N-treatments (Fig. II.4). The highest LD at the end of the growth period was obtained without NH_4^+ in the N treatment, however, it was only significantly different with LD of plants grown under 100% NH_4^+ in the N-treatment (Fig. II.4).

II.4. Discussion

II.4.1. General visual symptoms and responses

Brassica napus responded to limited N supply with a reduction in biomass production characterized by plants with short, thin main stems and few branches (Holmes, 1980) and a reduction in leaf area expansion (Ogunlela et al., 1989). The ontogeny of *B. napus* is also sensitive to N deficiency, with plants showing a significant reduction in the time to reach stem elongation (Chapter IV), flowering (Holmes. 1980) and maturity (Thomas, 1984). The N-form ratios have also been found to affect the ontogeny of plants. Ammonium nutrition compared with nitrate nutrition generally leads to early flowering of crop plants (Grasmanis and Leeper, 1967; Green et al., 1973; Tsujita et al., 1974; Haynes and Goh, 1977). However, it can also promote more vegetative growth in the form of early leaves and stems in a crop plant such as wheat (Spratt and Gasser, 1970), and thus alters ontogeny.

In this study the absence of N deficiency symptoms as well as the absence of changes in the time to reach stem elongation and flowering growth stages in *B. napus* (Harper, 1973) indicated that the level of the N-treatments were not deficient for normal plant growth and did not affected ontogeny. The changes in SDW, LA and RGR growth parameters as well as the other plant parameters in this study, therefore, can be ascribed either to random variation and to the N-form ratios and/or not to the N-levels (ie. N-concentration) used during this study.

II.4.b. Plant growth responses

Changes in dry matter accumulation are common plant responses to changes in N-form in the growth medium (eg. Cox and Reisenauer, 1973; Warnecke and Barber, 1973; Reisenauer et al., 1982). Normally, dry matter accumulation is enhanced in the presence of mixed N ($NH_4^+ + NO_3^-$), however, NH_4^+ as the sole source of N has generally been shown to be toxic to most plants, resulting in reduced dry matter accumulation (eg. Gashaw and Mugwira, 1981; Shen et al., 1990; Kaiser and Lewis, 1991). Rice is an exception to this general rule (Ismunadji and Dijkshoorn, 1971). In this study, NH_4^+ as the sole source of N enhanced dry matter accumulation of *B. napus*, despite the fact that *B. napus* is known to be sensitive to NH_4^+-based fertilizers, particularly when applied close to the seed (Thomas, 1984). Ammonium is also toxic to growth of *B. napus* under hydroponic conditions if the pH of the nutrient solution is not buffered (Moroni, unpublished data). Most of the toxic effects of NH_4^+ in plants have been observed in nutrient solution experiments. Graham (1984) suggested simple solution cultures should rarely be used to select nutrient efficiency factors, which are influenced by the root-soil interface. Thus, the observation that NH_4^+ is toxic to *B. napus* may be an artifact of the hydroponic system. The enhanced growth of *B. napus* in the presence of NH_4^+ in this experiment may be the result of the differential reaction of N-forms with the soilless matrix (Tisdale and Nelson, 1975). For example, conversion of NH_4^+ to NO_3^- (nitrification) may have been more rapid under the warmer environmental growth conditions of the greenhouse than under colder soil conditions generally found early in spring (Tisdale and Nelson, 1975). Also, NH_4^+ may be more tightly held by the soiless matrix and thus its toxic effect on *B. napus* may have been reduced (Tisdale and Nelson, 1975). In summary, the results obtained in this study indicated that dry matter accumulation of *B. napus* is enhanced when both forms of N were present in the growth medium.

II.4.c. Plant growth parameters

The observed variation in RGR induced by N-form ratios (Table II.1) can be analyzed in more detail by inspecting its components (Lambers et al., 1990). Relative growth rate can be divided into two components: RGR = NAR × LAR, the net assimilation rate (NAR, g SDW m-2 LA day-1) and the leaf area ratio (LAR; Lambers et al., 1990). Thus, differences in RGR are not necessarily due to variation in NAR, the efficiency with which the leaves acquire carbon, but may also reflect the relative amount of biomass a plant invests in LA (Lambers et al., 1990). Leaf area ratio (LAR) varies widely between species and depends on environmental conditions such as N supply (Sage and Pearcy, 1987). In this study, the observed reduction of LAR over the growth period was a reflection of the ontogenic switch from the vegetative stage to the stem elongation stage (Harper, 1973).

The LAR can be further divided into two components: leaf fraction and specific leaf area (ie. LAR = LF × SLA; Lambers et al., 1990). Leaf fraction is the ratio of leaf dry weight to total shoot dry weight and is a measure of the proportion of the total assimilate retained by the foliage (Causton and Venus, 1981). Specific leaf area is the ratio of leaf area to leaf dry weight and indicates very broadly the kind of leaf structure produced from the available dry material. A high SLA indicating thin leaves of relatively large area, and *vice versa* (Causton and Venus, 1981).

According to Loomis and Connor (1992) specific leaf weight (SLW, the reciprocal of SLA) is a useful index of leaf structure related to thickness and density, and hence to the balance between radiation capture and provision for diffusion through the air spaces within the leaf (Loomis and Connor, 1992). Furthermore, leaf net photosynthesis is often strongly correlated with SLW (Pearce et al., 1969).

Leaf density (LD) is the ratio of leaf fresh weight (LFW) to leaf dry weight (LDW). It provides a measure of the level of succulence or fleshiness of the leaf structure.

Therefore, variation in LAR may be due to a difference in "investment" in leaf biomass (ie. a difference in LF) or to a difference in leaf morphology (ie. difference in SLA; Lambers et al., 1990; Dijkstra, 1990).

In this study, the major cause of the LAR differences can be attributed to changes in the SLA and not LF. Both LF and SLA, however, are not entirely independent parameters (Lambers et al., 1990). For example, an extra investment of biomass per unit LA (all other parameters being equal) will decrease SLA, but increase LF (Lambers et al., 1990). In summary, the presence of NH_4^+ in the growing medium induced the development of plants with less dense (high SLA) leaves relative to plants growing in only NO_3^-.

II.4.d. Change of preference for N forms

The observed changes in ranking of the N-treatment effects on LAR, SLA and LD changed throughout the growing period of this study and may have been caused by the large random variation observed in this study. However, the possibility exists that the observed changes may be attributed to the changes in preference of *B. napus* for N forms, although there was not sufficient precision in this experimental design to critically estimate the changes in ranking among N treatments over the growth period. Plant species differ in response to N forms over the course of developmental growth (McKee, 1962; Dibb and Welch, 1976). Thus, it would be useful to determine if the observations of changes in ranking amongst N treatments were due to changes in plant stage sensitivity to different N-forms, and therefore to N-ratios, at different stages of the growing period, or as a result of random variation.

II.5. Conclusions

Growth of *B. napus* was maximized when a portion of the total N was supplied as NH_4^+ with SLA being the major growth parameter affected. However, there did not appear to be an optimum $NH_4^+:NO_3^-$ ratio for maximum growth. It is therefore suggested that when selecting N-efficient *B. napus* genotypes, N-treatments should contain a portion of NH_4^+ to achieve maximum growth. If the experimental protocol is designed with small pots and the N treatment is done at seeding then, a 10% of would be more appropriate to avoid conversion toxicity.

II.6. References

Causton D R and Venus J C 1981 The biometry of plant growth. Edward Arnold (Publishers), London, UK. 307 p.

Clarkson D T, Jones L H P and Purves J V 1992 Absorption of nitrate and ammonium ions by *Lolium perenne* from flowing solution cultures at low root temperatures. Plant Cell Environ. 15, 99-106.

Cox W J and Reisenauer H M 1973 Growth and ion uptake by wheat supplied nitrogen as nitrate, or ammonium, or both. Plant Soil 38, 363-380.

Dibb D W and Welch L F 1976 Corn growth as affected by ammonium vs. nitrate absorbed from soil. Agron. J. 68, 89-93.

Dijkstra 1990 Cause and effect of differences in specific leaf area. *In* Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants. Eds. H Lambers, M L Cambridge, H Konings and T L Pons pp 125-140. SPB Academic Publishing bv, The Hague, The Netherlands.

Errebhi M and Wilcox G E 1990 Tomato growth and nutrient uptake pattern as influenced by nitrogen form ratio. J. Plant Nutr. 13, 1031-1043.

Fried M, Zsoldos F, Vose P B and Shatokhin I L 1965 Characterizing the NO_3 and NH_4 uptake process of rice roots by use of ^{15}N labeled NH_4NO_3. Physiol. Plant. 18, 313-320.

Gardner F P, Pearce R B and Mitchell R L 1985 Physiology of crop plants. Iowa State University Press, Ames, USA. 327 p.

Gashaw L and Mugwira L M 1981 Ammonium-N and nitrate-N effects on the growth and mineral compositions of triticale, wheat, and rye. Agron. J. 73, 47-51.

Gomez K A and Gomez A A 1984 Statistical procedures for agricultural research. John Wiley & Sons, New York, USA. 680 p.

Goyal S S, Lorenz O A and Huffaker R C 1982 Inhibitory effects of ammoniacal nitrogen on growth of radish plants: 1. Characterization of toxic effects of NH_4^+ on growth and its alleviation by NO_3^-. J. Amer. Soc. Hort. Sci. 107, 125-129.

Graham R D 1984 Breeding for nutritional characteristics in cereals. Adv. Plant Nutr. 1, 57-102.

Grasmanis V O and Leeper G W 1967 Ammonium nutrition and flowering of apple trees. Aust. J. Biol. Sci. 20, 761-767.

Green J L, Holley W D and Thaden B 1973 Effects of the $NH_4^+:NO_3^-$ ratio, chloride, N-serve, and simazine on carnation flower production and plant growth. Proc. Fla. State Hortic. Soc. 86, 383-388.

Harper F R 1973. A key to standardize the description of growth stages in turnip rape, *Brassica campestris*. Can. Plant Dis. Surv. 53, 93-95.

Haynes R J and Goh K M 1977 Evaluation of potting media for commercial nursery production of container-grown plants. II. Effects of media, fertilizer nitrogen, and nitrification inhibitor on yield and nitrogen uptake of Callistephus chinensis (L.) Nees 'Pink Princess'. New Zealand Journal of Agricultural Research 20, 371-381.

Haynes R J and Goh K M 1978 Ammonium and nitrate nutrition of plants. Biol. Rev. 53, 465-510.

Holmes M R J 1980 Nutrition of the oilseed rape crop. Applied Science Publishers Ltd., London, UK. 158 p.

Ismunadji M and Dijkshoorn W 1971 Nitrogen nutrition of rice plants measured by growth and nutrient content in pot experiments. Ionic balance and selective uptake. Neth. J. Agric. Sci. 19, 223-236.

Jungk A 1970 Interactions between the nitrogen concentration (NH_4, NH_4NO_3, and NO_3) and the pH of the nutrient solution, and their effects on the growth and ion balance of tomato plants. Gartenbauwissenschaft 35, 13-28.

Kaiser J J and Lewis O A M 1991 The influence of nutrient nitrogen source on the growth and productivity of sunflower (*Helianthus annuus* var. Dwarf Sungold). S. Afr. Tydskr. Plantk. 57, 6-9.

Krstic B, Gebauer G and Saric M 1986 Specific response of sugar beet cultivars to different nitrogen forms. Z. Pflanzenernaehr. Bodenkd. 149, 561-571.

Lambers H, Freijsen N, Poorter H, Hirose T and Van der Werf A 1990 Analysis of growth based on net assimilation rate and nitrogen productivity. Their physiological background. *In* Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants. Eds. H Lambers, M L Cambridge, H Konings and T L Pons pp 1-17. SPB Academic Publishing bv, The Hague, The Netherlands.

Levene H 1960 Robust tests for equality of variances. *In* Contributions to probability and statistics. Eds. I Olkin, S G Ghurye, W Hoeffding, W G Madow and H B Mann pp 278-292. Standford University Press, Standford, USA.

Loomis R S and Connor D J 1992 Crop Ecology: productivity and management in agricultural systems. Cambridge University Press, Cambridge, UK. 538 p.

Macduff J H and Trim F E 1986 Effects of root temperature and form of nitrogen nutrition on nitrate reductase activity in oilseed rape (*Brassica napus* L.). Ann. Bot. 57, 345-352.

Macduff J H and Wild A 1989 Interactions between root temperature and nitrogen deficiency influence preferential uptake of NH_4^+ and NO_3^- by oilseed rape. J. Exp. Bot. 40, 195-206.

Magalhaes J R and Wilcox G E 1983 Tomato growth and mineral composition as influenced by nitrogen form and light intensity. J. Plant Nutr. 6, 847-862.

Marschener H 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press, London, UK. 889 p.

McKee H S 1962 Nitrogen metabolism in plants. Clarendon Press, Oxford, UK. 728 p.

Ogunlela V B, Kullmann A and Geisler G 1989 Leaf growth and chlorophyll content of oilseed rape (Brassica napus L.) as influenced by nitrogen supply. J. Agron. Crop Sci. 163, 73-89.

Pearce R B, Carlson G E, Barnes D K, Hart R H and Hanson C H 1969 Specific leaf weight and photosynthesis in alfalfa. Crop Sci. 9, 423-426.

Reiseneaur H M, Clement C R and Jones L H P 1982 Comparative efficiency of ammonium and nitrate for grasses. Proc. 9th Intl. Plant Nutr. Coll. 2, 539-544.

Sage R F and Pearcy R W 1987 The nitrogen use efficiency of C_3 and C_4 plants. I. Leaf nitrogen, growth and biomass partitioning in *Chenopodium album* and *Amaranthus retroflexus* (L.). Plant Physiol. 84, 954-958.

SAS Institute 1992 SAS/STAT User's Guide. Version 6. 4th ed. SAS Institute, Cary, North Carolina.

Shen L, Foster J G and Orcutt D M 1990 Influence of nitrate and ammonium on the growth and 2,4-diaminobutyric acid composition of flatpea (*Lathyrus sylvestris* L.). Plant Cell Environ. 13, 833-838.

Smith R L, Mills H A, Hoveland C S and Hanna W W 1990 Influence of ammonium:nitrate ratios on the growth and nitrogen uptake of pearl millet. J. Plant Nutr. 13, 541-553.

Spratt E D and Gasser J K R 1970 The effect of ammonium sulphate treated with a nitrification inhibitor, and calcium nitrate, on growth and N-uptake of spring wheat, ryegrass and kale. J. Agric. Sci., Cambr. 74, 111-117.

Taylor G J and Foy C D 1985 Mechanisms of aluminum tolerance in *Triticum aestivum* (wheat). IV. The role of ammonium and nitrate nutrition. Can. J. Bot. 63, 2181-2186.

Thomas P 1984 Canola growers manual. Canola Council of Canada. Saskatoon, Canada.

Tisdale S L and Nelson W L 1975 Soil Fertility and Fertilizers. 3rd ed. Macmillan Publishing Co., Inc., New York, USA. 694 pp.

Tsujita M J, Kiplinger D C and Tayama H K 1974 The effects of nitrogen nutrition, temperature, and light intensity on the growth, flowering, quality, and chemical composition of 'Indianapolis yellow' chrysanthemum. HortSci. 9, 294.

Xu Q F, Tsai C L and Tsai C Y 1992 Interaction of potassium with the form and amount of nitrogen nutrition on growth and nitrogen uptake of maize. J. Plant Nutr. 15, 23-33.

Warncke D D and Barber S A 1973 Ammonium and nitrate uptake by corn (*Zea mays* L.) as influenced by nitrogen concentration and NH_4/NO_3 ratio. Agron. J. 65, 950-953.

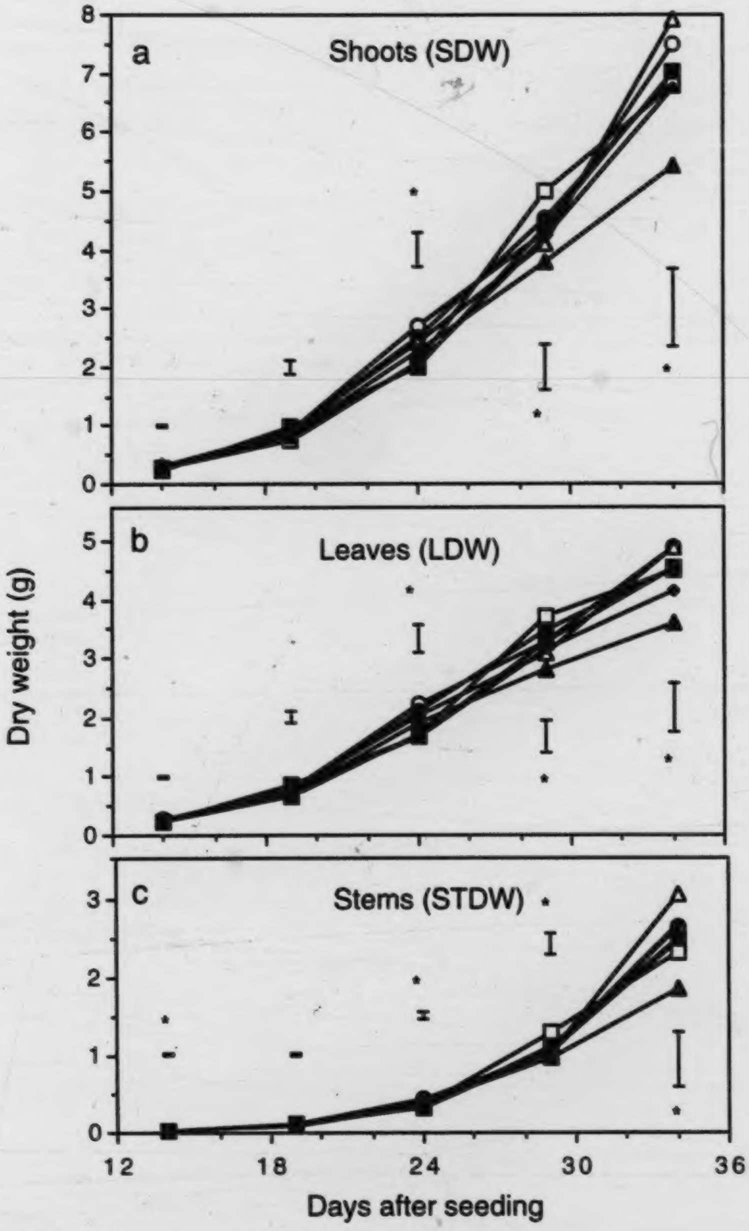


Fig. II.1. The effect of N-forms ratio (% NH_4:NO_3 ○100:0; ● 50:50; □ 40:60; ■ 30:70; △ 20:80; ◆ 10:90; ▲ 0:100) on dry weight accumulation of total shoots (a), leaves (b) and stems (c) of *Brassica napus* (L.) cv. Alto over time. Vertical bars represent LSD ($p \leq 0.05$).

* significant differences.

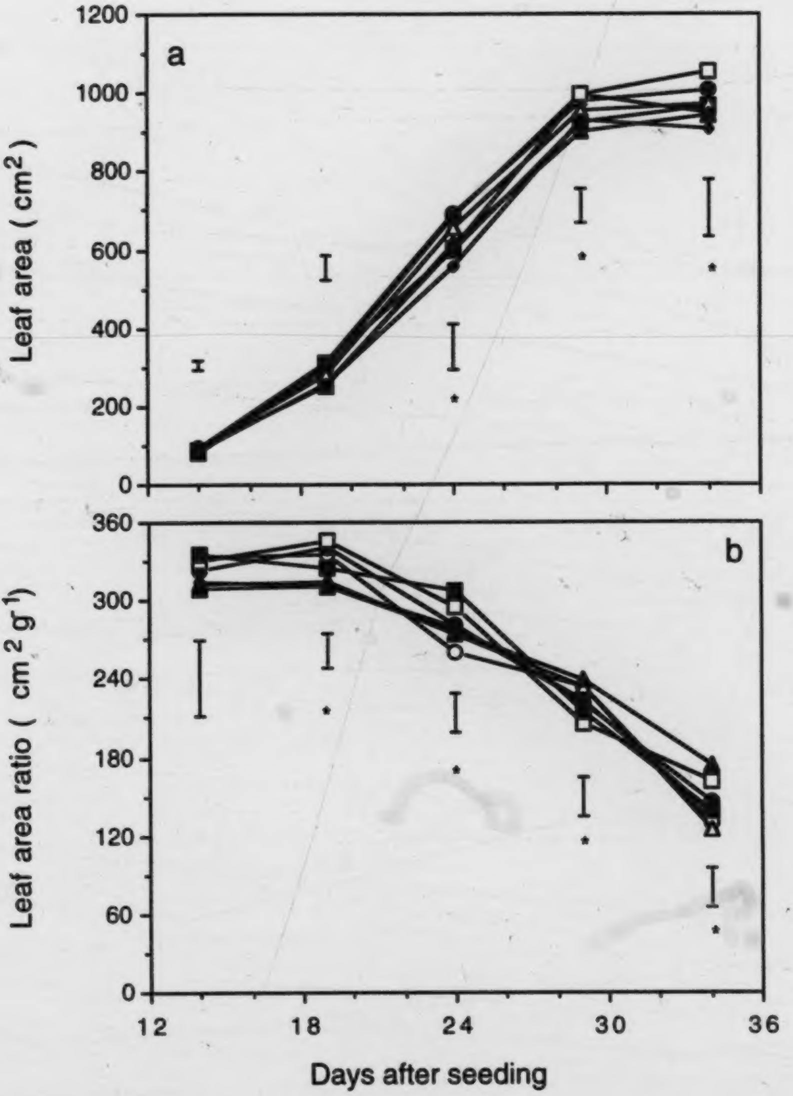


Fig. II.2. The effect of N-forms ratio (% NH_4:NO_3 ○ 100:0; ● 50:50; □ 40:60; ■ 30:70; △ 20:80; ◆ 10:90; ▲ 0:100) on leaf area (a) and leaf area ratio (b) of *Brassica napus* (L.) cv. Alto over time.
Vertical bars represent LSD ($p \leq 0.05$).
* significant differences.

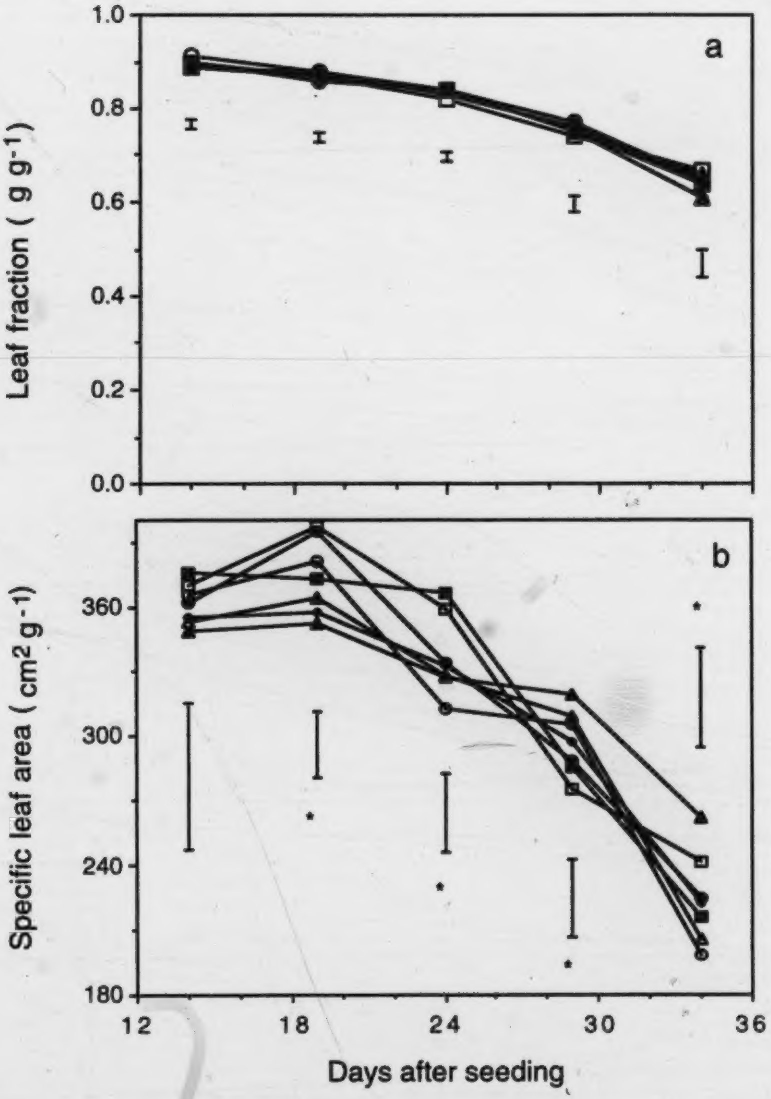


Fig. II.3. The effect of N-forms ratio (% NH_4:NO_3 ○ 100:0; ● 50:50; □ 40:60; ■ 30:70; △ 20:80; ◆ 10:90; ▲ 0:100) on leaf fraction (a) and specific leaf area (b) of *Brassica napus* (L.) cv. Alto over time.
Vertical bars represent LSD ($p \leq 0.05$).
* significant differences.

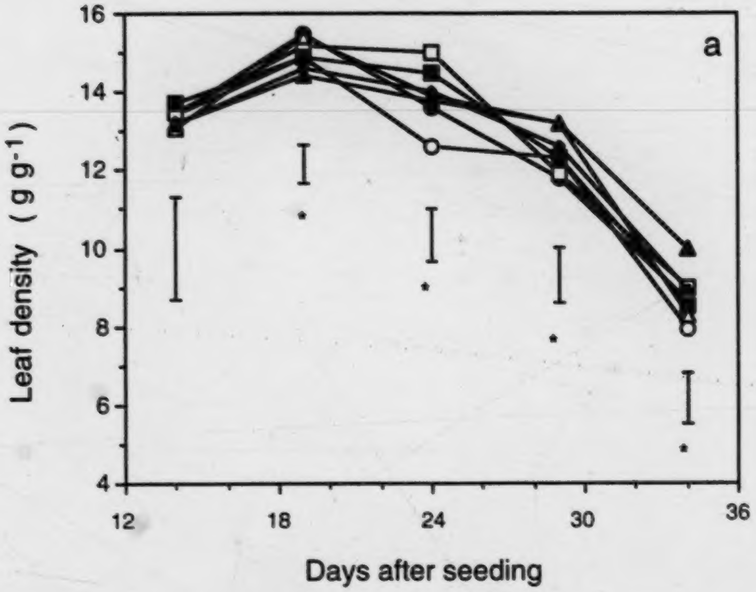


Fig. II.4. The effect of N-forms ratio (% $NH_4:NO_3$ ○ 100:0; ● 50:50; □ 40:60; ■ 30:70; △ 20:80; ◆ 10:90; ▲ 0:100) on leaf density (a) of *Brassica napus* (L.) cv. Alto over time.
Vertical bars represent LSD ($p \leq 0.05$).
* significant differences.

Table II.1. The effect of N-form ratio (NH_4^+:NO_3^-) on the relative growth rate (RGR) of rapeseed (*Brassica napus* L.) cv. Alto.

N-treatment (% NH_4^+)	RGR ($g\ g^{-1}\ day^{-1}$)
40	0.169 a†
100	0.168 a
30	0.165 a
20	0.162 ab
10	0.161 ab
50	0.160 ab
0	0.145 b

† Means followed by the same letter are not significantly different at the 5% level according to Duncans multiple range test.

CHAPTER III

The use of intraspecific grafts to evaluate the influence of the root and shoot systems on nitrogen efficiency in rapeseed (*Brassica napus* L.).

III.1. Introduction

Genotypic differences in nitrogen (N) efficiency of crop plants could result from differences in N-uptake and N-utilization of the shoots and roots or a combination of both. For example, iron efficiency in soybean has been found to be controlled by the root system (Brown et al., 1958), while in white clover, phosphorus efficiency has been shown to be controlled by the shoot system (Caradus and Snaydon, 1986). The effect of shoot and root systems in the expression of N-efficiency in rapeseed genotypes (*Brassica napus* L.) has not been studied. Identification of the relative influences of root and shoot systems on the expression of N-efficiency in a high N-requiring crop such as *B. napus* could be useful when developing N-efficiency screening methodologies.

The relative importance of shoot and root systems in controlling N-efficiency can be studied amongst plants of different genetic backgrounds by reciprocal scion/root grafting techniques. Several nutrient and plant species combinations have been studied using reciprocal grafting. For example, the technique has been used to study boron in artichokes and sunflower (Eaton and Blair, 1935), phosphorus in white clover (Caradus and Snaydon, 1986) and, iron in sunflower, cucumber (Romera et al., 1992), soybean (Brown et al., 1958) and tomato (Brown et al., 1971). This approach was also used to determine the effect of N fertility on shoot physiological parameters such as leaf area, chlorophyll content, CO_2 exchange and Rubisco concentration between burley and flue-cured tobacco (Crafts-Brandner et al., 1987a,b). Their study indicated that the root system did not influence the expression of physiological differences observed in the shoots (Crafts-Brandner et al., 1987a,b). This simple procedure appears to be a powerful technique to study mineral nutrition in plants and to determine the influence of shoot or root systems on the overall physiological performance of plants.

The objective of this study was to determine the relative influence of the shoot and root systems on N-efficiency parameters of *B. napus* genotypes which differ in the expression of N-efficiency (Chapter VI). Intraspecific reciprocal scion/root grafts between an N-efficient and three N-inefficient *B. napus* germplasm accessions were used for this study.

III.2. Materials and Methods

III.2.a. Plant culture and grafting technique

Four accessions of spring rapeseed (*Brassica napus* L.) were used in this study. Pera (Germany) was N efficient, while Bronowski (Poland), GSL-I (India) and Andor (University of Alberta, Canada) were found to be N inefficient when tested using a rapid N-efficiency screening test (Appendix 2; Table III.1). Seedlings were grown in a controlled environment chamber in 5×5 cm plastic inserts filled with a soil-free medium (Vermiculite + Peat Moss + Sand; 3:3:1 volume basis). A fertilizer mixture, containing normal levels of macro- and micro-nutrients, was mixed with the growth medium prior to potting. Intraspecific scion/root grafts consisting of: Pera×Bronowski, Pera×GSL-I and Pera×Andor were developed when seedlings were six to eight days old. PVC manifold pump tubing (I.D. 1.2-1.7 mm) was slit lengthwise and cut into approximately 1 cm lengths for holding the graft stocks. The hypocotyl of young seedlings were severed midway between the cotyledons and the soil surface using a slanting-cut with a sharp razor blade. The severed scion (*ie.* hypocotyl and cotyledons) was maintained in a water-filled vial until grafted to the hypocotyl of the root stock. A tubing section, of similar diameter to the hypocotyl of the root-stock, was slipped half-way over the root-stock and filled with distilled water. The scion portion of the hypocotyl was then inserted into the tubing and aligned to fit the root-stock slanting-cut by rotating it gently. A total of 816 grafts were performed.

Graft treatments consisted of non-grafted controls, grafted controls and intraspecific reciprocal grafts, and were designated according to shoot genotype/root genotype combinations. For example, for the reciprocal Pera×Andor combination, graft treatments consisted of Pera and Andor (non-grafted controls), Pera/Pera and Andor/Andor (grafted controls), and Pera/Andor and Andor/Pera (intraspecific reciprocal grafts). Therefore, a total of 14 graft treatment combinations were developed: three pairs of reciprocal grafts, four self grafts and the four non-grafted parental genotypes.

Grafted and non-grafted plants were kept for two to four days in a mist chamber at 100% relative humidity and a day/night temperature of 22/20°C. A mixture of fluorescent and incandescent light fixtures provided a low light intensity with a photosynthetic photon flux density (PPFD) of 280 ± 14 μmol m^{-2} s^{-1} for 16 h daily. Subsequently, the humidity was gradually reduced to ambient levels while the light intensity was gradually increased to a PPFD of 379 ± 23 μmol m^{-2} s^{-1}. The seedlings were watered regularly and fertilized with a weak concentration of 20-20-20 (NPK) fertilizer solution. Twenty four to twenty six days after grafting, plants were gradually hardened during 10 days before transplanting to the field (June 8, 1993) at the University of Alberta Research Station in Edmonton. Plants were spaced at a distance of 12 inches.

A random sample of graft treatments totaling 560 plants were used in the experiment. The remaining graft genotypes were randomly planted around the plot as border rows. After transplanting, plants were watered manually. Subsequently, plants were sprinkle irrigated when necessary. Air and soil temperatures for the duration of the experiment as well as N-levels of the soil were not determined.

III.2.b. Measurement of plant growth and efficiency parameters

The plant shoots were harvested after either 25 or 39 days of growth in the field. The plants (five per graft treatment) were cut at the ground surface and pooled. Variables measured were: shoot dry weight (SDW) and percent N content in the shoot (N%). Samples were dried to constant weight at 65° in a forced-air flow-oven, weighed and ground in a Wiley mill fitted with a 40 mesh screen (Arthur H. Thomas Co., Scientific Apparatus, Philadelphia, PA, USA). Percent nitrogen was measured by the combustion method using a LECO FP-2000 Nitrogen-Protein analyzer (LECO Corporation, St. Joseph, MI, USA). A homogeneous subsample of approximately 0.8-0.9g was used to determine N%. The following efficiency parameters were calculated:

Total N absorbed (TOTN) = $((((SDW \times N\%)/100)/14.0) \times 1000)$ (mmol^{-1} N),

Nitrogen use efficiency (NUE) = SDW/TOTN (g mmol^{-1} N) and

Efficiency of utilization (EU) = SDW$\times$(SDW/TOTN) (g^{2} mmol^{-1} N; Siddiqi and Glass, 1981).

III.2.c. Experimental design and statistical analysis

The experimental design was a split-plot design with 14 graft treatments, two harvest times, five plants per graft treatment and four replicates where graft treatments were randomly assigned to main plots and harvest times to subplots. Analysis of variance (ANOVA) were performed for SDW, TOTN, NUE and EU components. According to Levene's homogeneity test (Levene, 1960), as described by Milliken and Johnson (1984a), the variances for SDW and TOTN were non-homogeneous. Thus, ANOVA was performed on transformed data (ln). Comparisons between mean estimates for main and interaction effects were estimated by the PROC MIXED procedure available in the SAS/STATS statistical software (SAS Institute, 1992).

III.3. Results and Discussion

III.3.a. Grafting and graft treatment development

The grafting technique developed in this study was easier and faster than the "straw-band" technique (Bezdicek et al., 1972) commonly used for soybean, and is more suitable for seedlings with narrow and delicate hypocotyls like *B. napus*. Grafting did not appear to have any lasting detrimental effect on plant growth development, although some growth retardation relative to ungrafted plants was initially observed. In several cases, root growth from the scion at the scion/root graft union was noted. This appeared to be directly related to the length of time seedlings were kept under high humidity after grafting or when graft unions were too close to the growing medium. Further root growth from the graft unions was not observed amongst plants transplanted to the field. From a total of 816 grafts, 74 grafted plants died within a week and 60 died after trimming the root growth from the scion/root union, resulting in a survival rate of 84%. All grafted seedlings survived after 10 days of gradual hardening and the subsequent transplanting to the field. However, one of the replicates was lost due to an apparent soil contamination of the field and was not used in the analysis. Thus, ANOVA was performed on only three replicates.

Plant genotypes can be ranked as N-efficient or N-inefficient by the use of several nutrient efficiency definitions (Clark, 1990). In this study, total shoot dry weight (SDW), total N absorbed (TOTN), N use efficiency (NUE) and efficiency of utilization (EU; Siddiqi and Glass, 1981) were used as N-efficiency parameters to characterize and rank the four stock *B. napus* genotypes utilized in this study (Table II.1). The four genotypes were chosen amongst 111 accessions that were screened for N-efficiency under greenhouse conditions (Appendix 2; Table III.1). The genotypes used in this study were selected based on differences for N-efficiency, seed availability at the time and spring-type growth behavior. Based on half-normal statistical analysis (analysis not shown; Milliken and Johnson, 1984b) the Pera genotype expressed higher N-efficiency levels relative to the other three genotypes (Table III.1). Under field conditions Andor is a faster growing cultivar (earlier flowering) than the other cultivars. Andor was developed at the University of Alberta and is a genotype better adapted to Edmonton conditions compared to the other three genotypes. Analysis of variance indicated significant differences between graft treatments for all N-efficiency components (Table III.2.). However, for TOTN and NUE there were no graft treatment × harvest time interactions and thus, they were not explored further.

III.3.b. Comparisons between non-grafted controls: Pera versus Bronowski, GSL-I and Andor

Significant main effect differences between the non-grafted control Pera and the other three non-grafted control genotypes were detected for several of the N-efficiency parameters (Table III.3., set a). Shoot dry weight accumulation of Pera was significantly greater than Andor, and although Pera shoots absorbed significantly more N (TOTN) than Andor shoots, they did not differ significantly for NUE but, they did differ for EU. An interaction effect of graft treatment with harvest time was only detected for EU as a result of a significant difference shown at the second harvest (Table III.3., set a). The Pera genotype was not significantly different from Bronowski or GSL-I for either SDW or TOTN. However, there were significant differences between Pera and Bronowski for both NUE and EU and between Pera and GSL-I for EU. Interaction effects with harvest time between Pera and Bronowski were not detected but, interactions were detected for both SDW and EU in the Pera and GSL-I pair combination. Once again, the differences were the result of significant differences at only the second harvest and not both harvests. In all instances in which significant differences were detected Pera had a greater mean estimate. Thus, the nature of the N-efficiency differences between genotypes depended on the genotypic pair-wise combinations

These results show that differences for N-efficiencies between Pera and the other three genotypes measured in the preliminary screening under greenhouse conditions (Chapter VI, Table III.1.) were also expressed under field conditions (Table III.3., set a). Thus, the differences observed between parental stocks for N-efficient components provided useful genotypic variation for the study of the relative contribution of the shoot and the root systems of the N-efficiency parameters expressed in *B. napus* shoots by the grafting procedure.

III.3.c. Comparisons between non-grafted controls versus grafted controls

The grafting procedure *per se* may affect the physiological performance of the shoots which in turn may lead to changes in expression of N-efficiency parameters by plants. It is generally accepted that vascular continuity between scion and stock is a prerequisite for a successful graft. Translocation of assimilates can be restricted or blocked at the graft union by poor xylem and phloem connections as well as by enhanced deposition of starch in the graft union (Silberschmidt, 1933; de Stigter, 1971; Moore and Walker, 1981). A poor graft therefore, may result in changes to the profile of the differences for N-efficiency parameters between Pera and the other three genotypes. Furthermore, it may also confound the effects of the shoot/root grafting combinations on the expression of N-efficiency components. A comparison between the non-grafted control treatments and grafted control treatments would determine whether grafting *per se* had an effect on the shoot performance of each genotype.

Significant main effect differences between the non-grafted control and the grafted control treatments for some, but not all the N-efficiency parameters were detected (Table III.3., set b). Pera was not affected by grafting for any of the N-efficiency parameters (Table III.3., set b). Andor, on the other hand, was significantly affected by grafting for all the N-efficiency parameters except for NUE (Table III.3., set b). Also, grafting affected Bronowski for EU while GSL-I was affected for TOTN. Thus, grafting significantly influenced the expression of some N-efficiency parameters in the graft control treatments, with Andor being the most affected. Where significant main effects were detected the grafted control treatment expressed a greater mean estimate (Table III.3., set b). It should be noted that this results do not suggest that in some cases grafting increases N-uptake and that in other cases grafting increases growth rate. A possible, and more simple, explanation of the observed differences can be attributed to a retardation of the development of grafted plants whereby the vegetative growth stage may have been extended resulting in a larger vegetative matter accumulation.

A possible cause of the greater main mean levels of SDW, TOTN, and EU between the grafted and non-grafted controls of Andor may be the result of its differential growth response under the field conditions at Edmonton for which Andor is better adapted than the other three genotypes. Also, as mentioned above, some retardation in the growth stage development was observed in the grafted control plants relative to the non-grafted control plants. The leaf number among diverse genotypes is fairly constant between N-treatments (Chapter VI; Appendix 2). Leaf size and the concomitant increase in mass accumulation is not. Therefore, a longer vegetative growth stage resulted in an increase in overall mass accumulation. This may have resulted in making comparisons between grafted and non-grafted control treatments at two slightly different growth stages. However, in general, grafting was not found to dramatically affect the N-efficiency parameters under study (Crafts-Brandner et al., 1987a; Kleese, 1968) however, Caradus and Snaydon (1986) working on the phosphorus nutrition of white clover found a significant effect of self-grafting on three of 20 plant characters measured including a reduction of relative growth rate. To properly sort out these interactions a repeat of the experiment under controlled greenhouse conditions is necessary. These conditions would eliminate the hardening and transplanting of the plants and would allow for treatments to be tested earlier in the growth stage of the plants.

III.3.d. Comparisons of grafted controls: Pera versus Bronowski, GSL-I and Andor

As shown above, the grafting procedure *per se* affected some N-efficiency parameters of the graft control treatments when compared to non-grafted control. These effects may result in the elimination of existing differences between genotypes that were observed in non-grafted control treatments (Table III.3., set a). To determine the extent of the N-efficiency differences between the genotypes, the grafted control Pera genotype was compared to the other three grafted control genotypes (Table 2, set c).

Only two of the 12 main effects between Pera and the other three genotypes were changed as a result of grafting (compare Table III.3., set a *vs* set c). The two changes due to the grafting procedure occurred between Pera and Andor for TOTN, in which the significant difference disappeared, and between Pera and GSL-I for NUE, in which a significant difference was gained. Also, in every case that significant main effects were detected between Pera and the other three genotypes, the mean N-efficiency estimates of Pera were always greater. Overall, the differences between the stock genotypes were not greatly affected by the grafting procedure. Thus, regardless of the negative or positive effect that grafting may have had on the performance of the shoot genotypes, there were sufficient significant differences among Pera and the three other stock genotypes such that the N-efficiency of Pera relative to the other three stock genotypes was not lost. These results, therefore, indicted that significant differences existed between the selected genotypes for determining the relative effects of root and shoots on the expression of N-efficiency of *B. napus*.

III.3.e. Effects of the root system of the N-inefficient stock genotypes on the shoot system of the N-efficient stock genotype

The influence of root genotypes on the expression of N-efficiency parameters of shoots can be measured by comparing between intraspecific graft treatments composed of a common shoot genotype grafted to different root genotypes. In this study, Pera shoots, used as the common shoot genotype, were grafted with the three other root genotypes (Pera/Andor, Pera/Bronowski and Pera/GSL-I). If the root system has a significant effect on the expression of an N-efficiency parameter of the Pera shoots then, significant differences for N-efficiency parameters between the Pera/Pera graft control and intraspecific graft treatments should be detected.

Main mean effect estimates of the Pera graft control (Pera/Pera) treatments were not significantly different than the intraspecific graft treatments of Pera shoots with the three other root genotypes for any N-efficiency parameter (Table III.3., set d). This indicated that the root of the three N-inefficient genotypes did not significantly affect the expression of the N-efficiency parameters expressed by the Pera shoots: (Table III.3., set d). However, when the intraspecific graft treatments (ie. Pera/Andor, Pera/Bronowski and Pera/GSL-I) were compared with each of the graft control treatments namely: Andor/Andor, Bronowski/Bronowski and GSL-I/GSL-I, respectively, there were significant differences for only two N-efficiency parameters (Table 2, set f). The intraspecific graft treatment Pera/Andor was significantly higher than the graft control treatment Andor/Andor for EU while the intraspecific graft treatment Pera/Bronowski was significantly higher than the graft control treatment Bronowski/Bronowski for NUE. There were no graft treatment and harvest time interactions in these two sets of comparisons.

As discussed above (III.3.d) six main effects were significantly different between the graft control Pera/Pera and the three other graft control genotypes (Table III.3., set c). These significant main effect differences would be expected between the comparisons in set f if the roots from the extraneous genotypes are not significantly influencing the expression of phenotype in the shoots of Pera. However, as noted above, only two of the six expected remained significant, the other disappeared. Furthermore, the remaining six main effect comparisons remained the same (ie. no significant differences).

The observed loss of significant differences amongst four of the six N-efficiency parameters expected to be different (Table II.3., set c *vs* set f) may be the result of a relative reduction of the expression of the N-efficiency levels of Pera shoots caused by the extraneous root systems or a relative increase in the expression of N-efficiency levels of the grafted controls. It might also be the resulting effect of a negative influence on the root systems by the Pera shoots. The results indicated that there was a reduction in the levels of main effects for N-efficiency components of grafted controls shoots, there was not an increase in the N-efficiency component levels of the Pera shoots. Earlier, we noted that an increase in the levels of the main effects of the graft controls occurred because of grafting *per se*. Although that did not change the relative significant differences between Pera and the other three genotypes, the increase in levels might have changed sufficiently so that compared to the intraspecific graft treatments, significant differences were detected. However, taken together, these two sets of comparisons indicated that the root system from the N-inefficient genotypes did not influence the performance of the N-efficient Pera shoots. Pera shoots may have influenced the root systems of the N-efficient roots systems. Since root systems were not compared, this hypothesis needs further study.

III.3.f. Effects of the root system of the N-efficient genotype on the shoot system of the N-inefficient genotypes

The effect of the Pera root genotype on the shoots of the other three genotypes were determined by maintaining the Pera root system constant and altering the genotype of the shoot system. There were no significant differences for any N-efficiency parameter when the intraspecific graft treatment of the three shoot genotypes with the Pera root stock genotype were compared with the grafted control treatments Andor/Andor, Bronowski/Bronowski, and GSL-I/GSL-I (Table III.3., set g). These results were as expected. Also, there were no graft treatment and harvest time interactions. However, as expected, when the intraspecific graft treatments were compared with the graft control treatment Pera/Pera, significant differences for several of the N-efficiency parameters were observed (Table III.3., set e). Also, there were no graft treatment and harvest time interactions. Except for TOTN between the Andor/Pera intraspecific graft treatment and the Pera/Pera graft control treatment, all other N-efficiency parameters responded similarly to comparisons between grafted control Pera/Pera and the grafted control treatments Andor/Andor, Bronowski/Bronowski, and GSL.-I/GSL-I (Table 2, set b). In other words, the levels of N-efficiency parameters of the N-inefficient shoot genotypes were not significantly affected when attached to the root system from the N-efficient Pera. The resulting difference between the Pera shoot relative to the three other shoots when both were attached to the common Pera roots, was due to higher main effect estimates of the expression of the N-efficiency components of Pera shoots relative to the others and *vice versa*.

The Pera roots, therefore, appeared not to have an influence on the expression of the N-efficiency phenotype in shoots. The shoots of the N-inefficient genotypes appear to control their own level of expression of N-efficiency parameters. The shoot systems are of primary importance in effects to increase the level of N efficiency in plants. These sets of contrasts (Table III.3., sets d,e,f and g) demonstrate that the expression of N-efficiency parameters in *B. napus* shoots appear to be controlled by the physiology of the shoots and not the root systems. In the only other study in which N nutrition has been studied by intraspecific grafting (ie. shoot genotype of tobacco cultivars), the shoot was also found to be the controlling factor (Crafts-Brandner et al., 1987a). Furthermore, these results support a recent report indicating that the shoot controls nitrate uptake rates in *B. napus* (Laine et al., 1995).

III.4. Conclusions

Taken together, the results of this study provide evidence that the root system has a relatively small influence on the expression of N-efficiency components in shoots of *B. napus* genotypes differing in N-efficiency when grown under field conditions. If not all, a large portion of the expression of N-efficiency components in the N-efficient Pera is due to the shoot stock genotype. These results give credence to the hypothesis that the shoot system, and not the root system, is the major factor in determining N-efficiency components in *B. napus* genotypes. Nevertheless, confirmation of this conclusion requires further study as well as the testing of other *B. napus* genotypes.

III.5. References

Bezdicek D F, Magee B H and Schillinger J A 1972 Improved reciprocal grafting technique for soybeans (*Glycine max* L.). Agron. J. 64, 558.

Brown J C, Chaney R L and Ambler J E 1971 A new tomato mutant inefficient in the transport of iron. Physiol. Plant. 25, 48-53.

Brown J C, Holmes R S and Tiffin L O 1958 Iron chlorosis in soybeans as related to the genotype of rootstock. Soil Sci. 86. 75-82.

Caradus J R and Snaydon R W 1986 Plant factors influencing phosphorus uptake by white clover from solution culture. III. Reciprocal grafting. Plant Soil 93, 175-181.

Clark R B 1990. Physiology of cereals for mineral nutrient uptake, use, and efficiency. *In* Crops as enhancers of nutrient use. Eds. V C Baligar and R R Duncan pp 131-209. Academic Press, San Diego, USA.

Crafts-Brandner S J, Sutton T G and Sims J L 1987a Root system genotype and nitrogen fertility effects on physiological differences between burley and flue-cured tobacco. II. Whole plant. Crop Sci. 27, 1219-1224.

Crafts-Brandner S J, Leggett J E, Sutton T G and Sims J L 1987b Effect of root system genotype and nitrogen fertility on physiological differences between burley and flue-cured tobacco. I. Single leaf measurements. Crop Sci. 27, 535-539.

Eaton F M and Blair G Y 1935 Accumulation of boron by reciprocally grafted plants. Plant Phys. 10, 411-424.

Kleese R A 1968 Scion control of genotypic differences in Sr and Ca accumulation in soybeans under field conditions. Crop Sci. 8, 128-129.

Laine P, Ourry A and Boucaud J 1995 Shoot control of nitrate uptake rates by roots of *Brassica napus* L.: Effects of localized nitrate supply. Planta 196, 77-83.

Levene H 1960 Robust tests for equality of variances. *In* Contributions to probability and statistics. Eds. I Olkin, S G Ghurye, W Hoeffding, W G Madow and H B Mann pp 278-292. Stanford University Press, Stanford, USA.

Milliken G A and Johnson D E 1984a Analysis of Messy Data. Volume I: Designed experiments. Van Nostrand Reinhold Company, New York, USA. 473 p

Milliken G A and Johnson D E 1984b Analysis of Messy Data. Volume II: Nonreplicated experiments. Van Nostrand Reinhold Company, New York, USA. 199 p

Moore R and Walker D B 1981 Studies of vegetative compatibility-incompatibility in higher plants. II. A structural study of an incompatible heterograft between

Sedum telephoides (Crassulaceae) and *Solanum pennellii* (Solanaceae). Am. J. Bot. 68, 831-842.

Romera F J, Alcantara E and de la Guardia M D 1992 Role of root and shoots in the regulation of the Fe efficiency responses in sunflower and cucumber. Physiol. Plant. 85, 141-146.

SAS Institute 1992 SAS/STAT User's Guide. Version 6. 4th ed. SAS Institute, Cary, North Carolina, USA.

Siddiqi M Y and Glass A D M 1981 Utilization index: a modified approach to the estimation and comparison of nutrient utilization efficiency in plants. J. Plant Nutr. 4, 289-302.

Silberschmidt K 1933 Beitrage zur Kenntnis der Stoffwechselgemeinschaft zwischen Pfropfpartnern. Planta 19, 729-780.

Stigter H C M de 1971 Some aspects of the physiological functioning of the graft muskmelon/*Cucurbita ficifolia*. I. Melon/*Cucurbita* versus Cucumber/*Cucurbita*: differences in behaviour due to difference in degree of compatibility. Z. Pflanzenphysiol. 65, 223-231.

Table III.1. The effect of deficient (1.5 mmol) and sufficient (5.1 mmol) nitrogen supply on the expression of N-efficiency parameters of four *Brassica napus* (L.) genotypes. Partial data set obtained from Chapter 6. (Full results of both screenings were tabulated in Appendix 2).

	B. napus genotypes			
Nitrogen efficiency parameters†	Bronowski (Poland)	G.S.L.-I (India)	Andor (U of A)	Pera (Germany)
$SDW_{1.5}$ (g)	0.78	0.60	0.54	1.54
$SDW_{5.1}$ (g)	0.93	0.87	0.73	2.41
$TOTN_{1.5}$ (mmol N)	1.68	1.64	1.74	1.81
$TOTN_{5.1}$ (mmol N)	4.24	4.05	3.59	4.48
$NUE_{1.5}$ (g mmol^{-1} N)	0.47	0.36	0.31	0.85
$NUE_{5.1}$ (g mmol^{-1} N)	0.22	0.22	0.21	0.54
$EU_{1.5}$ (g^2 mmol^{-1} N)	0.36	0.22	0.17	1.30
$EU_{5.1}$ (g^2 mmol^{-1} N)	0.21	0.19	0.15	1.29
AE (g mmol^{-1} N)	0.04	0.08	0.05	0.24
AR (%)	71.20	67.00	51.40	74.30
PE (g mmol^{-1} N)	0.06	0.12	0.10	0.33

† Physiological efficiency (PE) = $(SDW_{5.1} - SDW_{1.5})/(TOTN_{5.1} - TOTN_{1.5})$

Agronomic efficiency (AE) = $(SDW_{5.1} - SDW_{1.5})$/net N applied)

Apparent recovery (AR) = $((TOTN_{5.1} - TOTN_{1.5})$/net N applied)) × 100

Table III.2. Analysis of variance (ANOVA) for the expression of N-efficiency parameters of graft treatments of *Brassica napus* (L).

Source	Shoot dry weight (SDW)	Total absorbed nitrogen (TOTN)	Nitrogen use efficiency (NUE)	Efficiency of utilization (EU)
Harvests	**	**	ns	**
Graft treatments	***	***	**	***
Harvests × Graft treatments	**	ns	ns	***

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively. ns not significant.

Table III.3. Comparisons of main and interaction estimates effects between graft treatments of *Brassica napus* (L). for the shoot expression of N-efficiency parameters.

Comparison between graft treatments	Shoot dry weight						Total nitrogen absorbed			Nutrient use efficiency			Efficiency of utilization					
	means		main	inter	Harv1	Harv2	means		main	means		main	means		main	inter	Harv1	Harv2
Set a																		
Pera *vs* Andor	16.8	9.7	***	ns	-	-	42.2	26.0	***	0.42	0.39	ns	6.9	3.7	***	***	ns	***
Pera *vs* Bron.	16.8	16.8	ns	ns	-	-	42.2	48.7	ns	0.42	0.37	*	6.9	6.0	*	ns	-	-
Pera *vs* GSL-I	16.8	13.1	ns	***	ns	**	42.2	33.3	ns	0.42	0.40	ns	6.9	5.3	***	***	ns	***
Set b																		
Pera *vs* Pera/Pera	16.8	15.2	ns	ns	-	-	42.2	37.3	ns	0.42	0.42	ns	6.9	6.3	ns	*	ns	**
Andor *vs* Andor/Andor	9.7	14.2	***	ns	-	-	26.0	37.6	***	0.39	0.39	ns	3.7	5.4	***	*	ns	***
Bron. *vs* Bron./Bron.	16.8	14.1	ns	ns	-	-	48.7	39.9	ns	0.37	0.35	ns	6.0	5.1	*	*	ns	**
GSL-I *vs* GSL-I/GSL-I	13.1	15.4	ns	ns	-	-	33.3	45.3	***	0.40	0.37	ns	5.3	5.4	ns	ns	-	-
Set c																		
Pera/Pera *vs* Andor/Andor	15.2	14.2	*	ns	-	-	37.3	37.6	ns	0.42	0.39	ns	6.3	5.4	*	ns	-	-
Pera/Pera *vs* Bron./Bron.	15.2	14.1	ns	ns	-	-	37.3	39.9	ns	0.42	0.35	**	6.3	5.1	**	ns	-	-
Pera/Pera *vs* GSL-I/GSL-I	15.2	15.4	ns	ns	-	-	37.3	45.3	ns	0.42	0.37	*	6.3	5.4	*	*	ns	***
Set d																		
Pera/Pera *vs* Pera/Andor	15.2	15.0	ns	ns	-	-	37.3	35.7	ns	0.42	0.42	ns	6.3	6.6	ns	ns	-	-
Pera/Pera *vs* Pera/Bron.	15.2	14.6	ns	ns	-	-	37.3	38.4	ns	0.42	0.42	ns	6.3	5.8	ns	ns	-	-
Pera/Pera *vs* Pera/GSL-I	15.2	14.8	ns	ns	-	-	37.3	38.9	ns	0.42	0.38	ns	6.3	5.7	ns	ns	-	-
Set e																		
Pera/Pera *vs* Andor/Pera	15.2	12.3	**	ns	-	-	37.3	28.3	*	0.42	0.43	ns	6.3	5.5	*	ns	-	-
Pera/Pera *vs* Bron./Pera	15.2	15.4	ns	ns	-	-	37.3	46.2	ns	0.42	0.34	**	6.3	5.2	*	ns	-	-
Pera/Pera *vs* GSL-I/Pera	15.2	15.1	ns	ns	-	-	37.3	43.0	ns	0.42	0.36	*	6.3	5.4	*	ns	-	-
Set f																		
Andor/Andor *vs* Pera/Andor	14.2	15.0	ns	ns	-	-	37.6	35.7	ns	0.39	0.42	ns	5.4	6.6	**	ns	-	-
Bron./Bron. *vs* Pera/Bron.	14.1	14.6	ns	ns	-	-	39.9	38.4	ns	0.35	0.42	**	5.1	5.8	ns	ns	-	-
GSL-I/GSL-I *vs* Pera/GSL-I	15.4	14.8	ns	ns	-	-	45.3	38.9	ns	0.37	0.38	ns	5.4	5.7	ns	ns	-	-
Set g																		
Andor/Andor *vs* Andor/Pera	14.2	12.3	ns	ns	-	-	37.6	28.3	ns	0.39	0.43	ns	5.4	5.5	ns	ns	-	-
Bron./Bron. *vs* Bron./Pera	14.1	15.4	ns	ns	-	-	39.9	46.2	ns	0.35	0.34	ns	5.1	5.2	ns	ns	-	-
GSL-I/GSL-I *vs* GSL-I/Pera	15.4	15.1	ns	ns	-	-	45.3	43.0	ns	0.37	0.36	ns	5.4	5.4	ns	ns	-	-

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively. ns = not significant.

CHAPTER IV

Time-course response of rapeseed (*Brassica napus* L.) to nitrogen supply: Selection of sampling time when screening for nitrogen efficient germplasm.

IV.1. Introduction

The utilization of genotypes efficient in the uptake and utilization of nitrogen (N) in a high N-requiring crop such as rapeseed (*Brassica napus* L.) can be an effective way of reducing N pollution and crop inputs (eg. Gerloff, 1963; Vose, 1963; Gabelman, 1976; Clark and Duncan, 1991). However, research on the improvement of N-efficiency in *B. napus* has been limited (Yau and Thurling, 1986; Gerath and Schweiger, 1991; Thurling, 1993; Gerath and Balko, 1995). To select useful plant genetic variation for nutrient efficiency, a reliable screening technique, and clearly defined plant growth and nutrient efficiency measures for ranking genotypes are necessary (Devine, 1982; Devine *et al.*, 1990). A two N-level screening technique provides a rapid procedure for screening genotypes (*eg.* Caradus and Dunlop, 1979), however, sensitivities to nutritional deficiencies and toxicities change as the plant progresses through ontogeny (Marschener, 1995; Duncan and Baligar, 1990). Thus, sampling time is important when selecting N-efficient *B. napus* germplasm. The most appropriate sampling-time can be determined from time-course × N-dose response curves.

The selection of a sampling time during the course of a growth period depends on the plant components to be measured and on the N-efficiency definitions used for ranking genotypes. Amongst the 37 nutrient efficiency definitions which have been proposed the most commonly used definition is nutrient concentration in tissue (Clark, 1990). Another widely used definition is nutrient use efficiency, which is the reciprocal of nutrient concentration (Siddiqi and Glass, 1981). Efficiency of utilization has been proposed as a way of integrating the final biomass and its nutrient concentration (Siddiqi and Glass, 1981). Nitrogen efficiency definitions used in ranking genotypes at an early growth stage have typically been calculated using biomass accumulation rather than leaf expansion (Clark, 1990). In *B. napus*, leaf area and biomass accumulation prior to anthesis are important growth components that influence yield (Thurling, 1993). Furthermore, most of the N is absorbed prior to anthesis (Holmes, 1980). How these N efficiency components change over time needs to be examined prior to developing a screening technique.

The effects of N supply on plants include those arising as a result of N deficiency and those arising as a result of N sufficiency. Nitrogen deficiency subjects plants to stressful conditions in which stress tolerance can be assessed, while N sufficiency subjects plants to non-stressful conditions allowing maximum genetic potential to be expressed (Baker, 1994). By determining the differential effect of N deficiency and N sufficiency on N efficiency components, the selection of N levels for developing screening techniques can be simplified. Examination of time-course × N-dose response curves should indicate when a transitional response from N sufficient to N deficient condition occurs, and also in which N-treatment range the two N-levels should fall for screening purposes.

In the present work, the response of *B. napus* cv. Alto to eight N levels over a period of 35 days of growth was examined (a) to determine a sampling-time for estimating N efficiency, (b) to evaluate leaf area and shoot dry weight for calculating N efficiencies and (c) to characterize the transitional point between N sufficiency and N deficiency.

IV.2. Materials and Methods

IV.2.a. Plant material and growing conditions

Plants of rapeseed (*Brassica napus* L.) cv. Alto were grown under greenhouse conditions in 10×10 cm square plastic pots filled with a soil-free medium (Vermiculite + Peat Moss + Sand; 3:3:1 volume basis). Each pot contained an average of 322 ± 3 g of growing medium, (dry weight basis), containing 26 ± 0.2 mmol total N per pot. Seeds of cv. Alto contained an average of 9.98 mmol N per 1000-kernel weight (on an oil-free basis: 4.53 mmol N g^{-1} seed; 0.22 g seed $mmol^{-1}$ N). Plastic bags sealed at one end were inserted into each pot to prevent nutrient loss through the drainage holes. Two to four seeds were seeded per pot and seedlings were thinned to one per pot seven days after seeding (DAS).

A modified basal nutrient solution (Taylor and Foy, 1985) was added to each individual pot providing (mmol): K, 1.60; PO_4, 0.40; SO_4, 0.40; Mg, 0.60; Cl, 1.34; Mn, 8×10^{-3}; B, 2.4×10^{-2}; Zn, 2×10^{-3}; Cu, 6×10^{-4}; Na, 8.1×10^{-2}; Mo, 4×10^{-4}; EDTA, 0.04; Fe, 0.04. Iron was supplied as Fe-EDTA prepared from equimolar amounts of $FeCl_3$ and Na_2EDTA. All nutrients were supplied in solution, except for Ca, which was supplied as powdered $CaSO_4$ and mixed into the medium. A nitrogen (N) solution consisting of 90% N-NO_3^- and 10% N-NH_4^+ was used to provide eight N-treatments (*ie.* 0, 0.6, 1.5, 2.4, 3.3, 4.2, 5.1, 8.0 mmol per pot) corresponding to a range of 0 to 112 kg N ha^{-1}. An earlier experiment demonstrated the positive response of *B. napus* to N supply when N-NO_3^- and N-NH_4^+ were supplied as a mixture rather than alone (Chapter II). Measured volumes of all nutrients, including N-treatments, were added to each individual pot one DAS. Plants were watered regularly with distilled water and care was taken to prevent waterlogging.

Plants were grown under greenhouse conditions at a temperature of 20-23 °C and relative humidity of 60-90%. Natural light was supplemented with 400 watt, high pressure sodium lamps (Sylvania) attached to high intensity discharge fixtures providing a basal average photosynthetic photon flux density (PPFD) of 280 ± 14 µmol m^{-2} s^{-1} for 16 h at soil surface height daily reaching a total PPFD of 686 ± 54 µmol m^{-2} s^{-1} when days were sunny and clear.

IV.2.b. Measurement of plant growth and N efficiency parameters

Shoots were harvested individually, by cutting at the surface of the growing medium, at 12, 16, 20, 25, 30 and 35 DAS. Roots were not harvested, because an earlier experiment had shown that shoots were the principal plant parameter accounting for differences in N efficiency (Chapter III). Variables measured included leaf area (LA), shoot dry weight (SDW), and percent N content in the plant shoot (N%). Leaf area was measured on a LI-COR LI3100 Area Meter (LI-COR Inc., Lincoln, Nebraska, USA). Samples were dried to constant weight at 65 °C, weighed and ground in a Wiley mill fitted with a 40 mesh size screen (Arthur H. Thomas Co., Scientific Apparatus, Philadelphia, PA, USA). Percentage nitrogen was measured by the combustion method using a LECO FP-2000 Nitrogen-Protein analyzer (LECO Corporation, St. Joseph, MI, USA). A homogeneous subsample of about 0.2-0.9 g was used to determine N%.

Plant component ratios calculated consisted of leaf area ratio (LAR = LA/SDW). Efficiency components calculated consisted of total nitrogen absorbed (TOTN) = $((((\mathrm{SDW} \times \mathrm{N\%})/100)/14.0) \times 1000)$ (mmol^{-1} N), nitrogen use efficiency ($\mathrm{NUE}_{\mathrm{SDW}}$) = SDW/TOTN (g mmol^{-1} N) and efficiency of utilization ($\mathrm{EU}_{\mathrm{SDW}}$) = SDW (SDW/TOTN) ($\mathrm{g}^2$ mmol^{-1} N; Siddiqi and Glass, 1981). Nitrogen use efficiency and EU were also calculated for each N-treatment on a LA basis (ie. $\mathrm{NUE}_{\mathrm{LA}}$, $\mathrm{EU}_{\mathrm{LA}}$) by replacing LA for SDW in the equations.

IV.2.c. Calculation of points of inflexion of the curves

The DAS when the LA, and TOTN response curves for all N-treatments underwent their point of inflexion ($\mathrm{DAS}_{\mathrm{PI}}$) were determined by fitting a non-linear function to the raw data of each curve and estimating the corresponding parameters of the functions. The point of inflexion in a sigmoidal curve is the point at which the concavity changes from upward to downward, or from downward to upward (Swokowski, 1979). It is also where the absolute rate of change is maximal (Richards, 1959). Although data transformation (ln) is commonly used to fit a linear model to non-linear data, the non-linear model the Richards' function was more appropriate for those data (see Appendix 3 for a graphical comparison). A reparameterised Richards function (Richards, 1969) as described by Cromer *et al.* (1993) was fit to each replication of the LA and TOTN curves at each N-treatment as follows:

$$W(t) = W_x\left(1 + d e^{r(1+d)(t_0 - t)}\right)^{-(1/d)}$$

Where W_x is the asymptotic value of W for large t, t_0 is the time at which $W(t)$ undergoes its point of inflexion, r is the relative growth rate of $W(t)$ at t_0 and d determines the shape of the curve of W versus t so that the point of inflexion occurs further up the curve with larger d (Cromer *et al.*, 1993). The inflexion points of the SDW curves for the two lowest N-treatments (0.0 and 0.6 mmol N) were obtained using this technique. These curves were the only SDW curves which showed sigmoidal curves in response to N supply (Fig. 1,a).

A linear spline model consisting of two straight lines with estimated knot (Smith, 1979; SAS Institute, 1991) was used to estimate when the NUE_{SDW} curve underwent its point of inflexion at each replication for each N-treatment as follows:

$$NUE_{SDW} = \beta_0 + \beta_1\,DAYS + \beta_2\,DAYS_x + \varepsilon$$

where $DAYS_x = DAYS - Knot$ if $DAYS \geq Knot$

and $DAYS_x = 0$ if $DAYS < Knot$

Smith (1979) indicated that "splines" are generally defined to be piecewise polynomials of degree 'n' whose function values and first $(n-1)$ derivatives agree at points where they join. The abscissae of these joint points are called "knots". Thus, the knots occur at the approximate point of inflexion for these curves. The resulting piecewise polynomial linear equations estimated for fitting the lower section of the NUE_{SDW} curves were used to determine the value of the Y-axis (NUE_{SDW}) where the point of inflexion occurred.

IV.2.d. Experimental design and statistical analysis

The experimental design was a randomized complete block, consisting of eight N-treatments, six harvests, five plants per N-treatment and three replications for a total of 720 pots. Fitting of the functions to data were performed by the nonlinear procedure (NLIN) available in the SAS/STATS statistical software (SAS Institute, 1991; SAS Institute, 1992). Analysis of variance (ANOVA) were performed on the estimated points of inflexion and significance between means was determined by the LSD multiple range test (Gomez and Gomez, 1984). The estimated points of inflexion for LA, TOTN and NUE_{SDW} were correlated between each other (Spearman correlation) and regressed on the N-treatments (Gomez and Gomez, 1984; SAS Institute, 1991, 1992). Unless otherwise indicated, all levels of significance were at $p<0.05$.

IV.3. Results

IV.3.a. Visual growth responses to N supply

Differential N supply resulted in differential N deficiency symptoms and changes in the development of Alto over the 35 days of growth. Nitrogen deficiency symptoms such as chlorotic cotyledons, chlorotic and purplish leaves and reduction in plant growth (Marschener, 1995) became visible at about 20-22 DAS in 0.0 and 0.6 mmol N. Deficiency symptoms were observed on plants growing in 5.1 mmol N at the last harvest (35 DAS). Nitrogen deficiency symptoms were increasingly delayed with each increase in N treatment. Within each N treatment, the severity of N deficiency symptoms increased over time. The onset of N deficiency symptoms coincided with a reduction in the rate of leaf expansion (Fig. IV.1b) and N absorption (Fig. IV.2a) amongst N treatments. Inhibition of stem elongation was noticeable at 26-28 DAS on plants growing in 0.0, 0.6, 1.5 mmol N and was observed progressively up to 5.1 mmol N at the final harvest. Some plants grown in 0.0 mmol N flowered by 30 DAS. At the final harvest, some plants were flowering in 2.4 mmol N. Thus, continuous and progressive severity of N deficiency symptoms was accompanied by reductions in the length of each of the developmental growth stages observed in this experiment (rosette, stem elongation and flowering; Harper, 1973).

IV.3.b. Time-course responses of plant growth components to N supply

Significant differential responses of SDW and LA to N supply were observed, however LA expansion was reduced earlier than SDW growth to N supply (Fig. IV.1a, b). Only plants growing in 0.0 and 0.6 mmol N showed a significant reduction in SDW accumulation resulting in sigmoidal curves for SDW (Fig. IV.1a). No significant reduction was observed in SDW growth for the other N treatments. In contrast, LA increased sigmoidally across all N-treatments resulting in significant differences between N treatments at each harvest except for 20 DAS (Fig. IV.1b). Significant increases in LA at and above 30 DAS only occurred in the 4.2, 5.1 and 8.0 N treatments (Fig. 1b). The SDW and LA growth reductions were initiated at different DAS (Fig. IV.1a,b). At 0.0 and 0.6 mmol N the reduction in LA expansion was observed several days earlier than the reduction in SDW (Fig. IV.1a,b). Thus, LA was a more sensitive indicator of N deficiency than SDW.

The absorption of N by shoots followed sigmoidal curves over the entire growing period in all N treatments (Fig. IV.2a). All growth curves, except in 8.0 mmol N, exhibited an asymptote indicating N limitation (Fig. IV.2a). The point of inflexion of

TOTN, where the onset of the N absorption rate reduction occurred, was significantly correlated with N treatment (r = 0.91; Table IV.1). For each 1 mmol increase in N the onset of the reduced N absorption rate was delayed by 1.2 days(Table IV.1). The TOTN curves (Fig. IV.2a) showed more similarity to the LA curves than to the SDW curves (Fig. 1), further supporting the more sensitive nature of LA relative to SDW in response to N supply.

A relatively constant LAR of about 350-380 $cm^2\ g^{-1}$ was observed for the first three harvests followed by a sharp reduction in all N-treatments (Fig. IV.2b). In general, these reductions were similar in all N-treatments, but a significant N-treatment effect on LAR was detected at or above 25 DAS between the two lowest N-treatments (0.0 and 0.6 mmol N) and the highest N-treatment (8.0 mmol N; Fig. IV.2b). Hence, as plants developed, a proportionally smaller 'partitioning' of photosynthates was diverted toward leaf expansion than to biomass growth. Proportionally, the reduction of LA was higher than the reduction of SDW due to N deficiency (Fig. IV.1a,b; Fig. IV.2b). These effects increased progressively in severity with declining N supply and the longer time after seeding (Fig. IV.2b).

IV.3.c. Time-course responses of nitrogen efficiency parameters

Nitrogen use efficiency (NUE) is yield per unit N in the tissue and thus is the reciprocal of N concentration (Glass, 1990). During initial growth, a relatively constant NUE_{SDW} level of about 0.20 g $mmol^{-1}$ N was maintained by shoots (Fig. IV.3,a). This level did not differ significantly amongst the six highest N treatments but its duration did (Fig. IV.3,a). This steady NUE_{SDW} level was maintained for a longer period of time in the high N treatments than in the low N treatments. Coincidentally, this NUE_{SDW} level was similar to the NUE of Alto seeds (*ie.* 0.22 g $mmol^{-1}$ N, oil-free basis; Chapter V). Whether this level is of any physiological significance and/or a reflection of a homeostatic response characteristic of each genotype, remains to be tested. Wide variability among NUE_{LA} curves prevented curve fitting (Fig. IV.3,b). No constant NUE_{LA} was observed such as the one observed for NUE_{SDW} (Fig. IV.3a,b).

The overall NUE_{SDW} or NUE_{LA} levels maintained over the entire growth period were higher at low levels of N supply. Treatment effects were significantly magnified when NUE was calculated on a LA (Fig. IV.3b) rather than on a SDW basis (Fig. IV.3a). Plants grown in the two lowest N-treatments showed a maximum NUE_{LA} was reached (Fig. IV.3b) at the approximate time of LA reduction (Fig. IV.1b) concomitant with the termination of N absorption (Fig. IV.2a). The larger NUE levels were obtained under conditions of diminishing N availability as plants developed.

Efficiency of utilization (EU) is expressed as the product of total yield times NUE and emphasizes the contribution of the total plant yield (Glass, 1990; Siddiqi and Glass, 1981). Differential EU responses to N supply were observed over the entire growing period, however these responses varied if EU was calculated on a SDW (EU_{SDW}; Fig. IV.4a) or LA basis (EU_{LA}; Fig. IV.4b). The increase in EU_{SDW} over the course of the growth period resulted in significant differences between N-treatments at all but the final harvest (Fig. IV.4a). The maximum EU_{SDW} was attained in the 2.4 mmol N treatment during final harvest (Fig. IV.4a). Visible signs of a decline in EU_{SDW} were only observed for plants grown in 0.0 and 0.6 mmol N (Fig. IV.4a).

The effect of N supply on EU_{LA} resulted in a sigmoidal growth response although, not all curves reached an asymptote (Fig. IV.4b). A significant reduction in EU_{LA} at the three lowest N-treatments were observed at and above 25 DAS which became accentuated over time (Fig. IV.4b). Most of the available N would have been absorbed by this time in these three N treatments (Fig. IV.2A). Since this was accompanied by a concomitant reduction of leaf expansion (Fig. IV.1a) it is unlikely that EU_{LA} would have increased beyond these maximum levels (Fig. IV.4b). The maximum EU_{LA} declined with declining N supply. The EU_{LA} should not change beyond a certain time period but should increase in magnitude and reach the maxima at later dates (DAS) for each increment in N supply (Fig. IV.4b). The maximum EU_{LA} was attained earlier than the maximum EU_{SDW} for most N treatments.

IV.3.d. Determining the transition of non-stress to stress N environment

The DAS at which LA, TOTN, and NUE_{SDW} curves underwent their point of inflexion (DAS_{PI}) have been summarized in Table IV.1. For the SDW curves, the 0.0 and 0.6 mmol N treatments were the only ones where the response attributed a sigmoidal pattern and were the only ones used for fitting Richards function (Richards, 1969). For these curves, the DAS_{PI} occurred at 26 and 27 DAS, respectively. Significant N treatment effects on DAS_{PI} were determined for LA, TOTN and NUE_{SDW}, which resulted in a positive linear relation in response to increasing N supply (Table IV.1). The rate of increase of DAS_{PI} in response to N supply was in the order of 1 day per mmol (Table IV.1). Except for 0.0 and 4.2 mmol N, differences between the DAS_{PI} means among LA, TOTN and NUE at each N-treatment were not significant (Table IV.1). However, the

DAS_{PI} for TOTN curves were consistently lower than that for LA and NUE_{SDW} by an average of about 2 days (Table IV.1). The DAS_{PI} of SDW curves (ie. 26 and 27 DAS for the 0.0 and 0.6 mmol N treatment, respectively) occurred about nine days after the DAS_{PI} of TOTN and about six days after the DAS_{PI} of LA for the same N treatments. Also, significant correlations ($p<0.001$) amongst the DAS_{PI} for TOTN, LA and NUE_{SDW} were observed. The correlations (r) for DAS_{PI} between TOTN with LA and NUE_{SDW} were 0.95 and 0.90, respectively, and the correlation for DAS_{PI} between LA with NUE_{SDW} was 0.88. This indicated a close relationship between the point of inflexion for N uptake and the point of inflexion for LA expansion.

IV.4. Discussion

IV.4.a. Differential response of LA relative to SDW

When screening N efficient *B. napus* germplasm, the effect of all factors involved in the screening protocol need to be defined and evaluated (Clark, 1990) including both the growth stage and the growth components to be measured. Sampling times and the N treatments to which the plants will be subjected are also important. The shape of the crop at rosette stage of *B. napus* defines the overall final yield (Thurling, 1993). Thus, the rosette stage is potentially a useful growth stage at which to screen for N efficiency in *B. napus*. The rosette stage begins when the first normal leaf is unfolded and terminates when the stem begins to elongate (Harper, 1973). However, changes in sensitivity to nutritional deficiencies and toxicities as the plant goes through developmental stages (Duncan and Baligar, 1990) would confound the selection of both the sampling time and N treatments. Describing the different responses induced by sampling-time × N-treatment combinations on growth components is therefore critical when developing a screening technique.

Leaf expansion and increases in shoot biomass are the main growth components of the rosette stage. Shoot dry weight is one of the most common growth components used to estimate N efficiency at an early growth stage (Clark, 1990). Still, there is evidence to suggest LA is a sensitive parameter to N stress and thus useful for determining N efficiency (Ogunlela et al., 1989). In this study, N deficiency symptoms and the shift from vegetative to reproductive stage occurred earlier under conditions of lower initial N supply. The resulting effect was in an earlier reduction of leaf expansion relative to biomass growth, indicative of the greater sensitivity of leaf expansion to N supply. Furthermore, these growth responses coincided with a transition from an environment of N sufficiency to N deficiency resulting from N depletion (Fig. IV.2a). Leaf expansion in *B. napus* is responsive to N supply (Allen et al., 1971; Allen and Morgan, 1972; Rood and Major, 1984; Ogunlela et al., 1990). The high responsiveness of LAI to increased N supply (Scott et al., 1973) has a strong positive association with seed yield (Allen and Morgan, 1975) during the early stage of reproductive development. Similarly, plant dry weight at anthesis is responsive to N supply in spring type (Thurling, 1974) and winter type (Mendham and Scott, 1975) rapeseed and has been closely correlated with seed yield. Thus, both LA and SDW are important in the final yield of *B. napus*. Two possible causes, either separately or in combination, may account for the differential response of LA relative to SDW and the concomitant changes in growth stages; a direct N deficiency causes inhibition of leaf expansion and/or the acceleration of the developmental stages from vegetative to reproductive. The reduction in leaf expansion by N deficiency may result from a reduction in cell division (MacAdam *et al.*, 1989), reduced cell size (McDonald, 1989) or a combination of both. The acceleration of developmental stage from vegetative to reproductive may result from a plastic response to stress inducing early flowering and sustaining reproductive allocation as a proportion of the biomass (Grime, 1989). This is common in crop plants under low fertility conditions, and results in a time phase reduction for each growth stage (Stoskopf, 1981). Thus, LA and SDW are responsive parameters useful for determining N efficiency components in *B. napus* germplasm.

Stress induced by N deficiency and its effect on the reduction of plant growth was detected earlier when estimated by LA rather than by SDW. The resulting differential effects on LA and SDW growth are reflected in the growth components TOTN and LAR as well as in the N efficiencies NUE and EU. Whether the differential responses between LA and SDW differ amongst *B. napus* genotypes and can be potentially useful in selecting N efficient germplasm, remains to be tested.

IV.4.b. Transition from N sufficiency to N deficiency

The combination of sampling-time and N-treatment will determine the nature of the selecting N-environment and the nature of the N efficiency responses observed. An N-deficient environment would subject plants to stressful conditions in which stress tolerance can be assessed while an N-sufficient environment would subject plants to non-stressful conditions in which maximum genetic potential can be assessed (Baker, 1994). Thus, N-efficiency estimates in either N environment would be a reflection of different and distinct characteristics of a genotype for N-efficiency. The interaction of both sampling time and N-treatment would therefore define the level of N stress to which plants would be subjected. Characterization and selection of the type of growing environment as affected by the N-level × time combination is therefore important.

The inflexion point of a growth curve is the time transitional phase at which the rate of growth is maximal and where a change from increasing to decreasing rate occurs (Richards, 1969). The inflexion point in a growth curve indicates a transition phase from N sufficiency to N deficiency or from a non-stressful to a stressful N environment and can be used to characterize the growing environment of the plants. In this study, this rate change can be attributed to the depletion of N in the growing medium since all environmental variables (*eg.* temperature, light, nutrients) were maintained relatively constant and in sufficient supply over the growing period. A change in N supply elicited changes in growth and N efficiency components (Fig. IV.1, IV.2, IV.3, IV.4). Thus, any period before the inflexion point indicated a non-stressful N level while any period after the inflexion point indicates a time of N stress.

IV.5. Conclusions

Caution should be exercised when selecting N-levels × sampling-time for screening N efficient germplasm. Minimizing growth stage differences is paramount if comparisons among genotypes are to be meaningful. For a two N-level screening of *B. napus* germplasm a growth period of 25-30 DAS will provide a sufficient time interval for plants to reach the late rosette stage. Both leaf area and shoot biomass may be useful growth components when ranking *B. napus* genotypes for N efficiency and may be used in a plant breeding selection index. Also, selection of N treatments at or below 1.5 and at or above 5.1 mmol N would provide a stressful and non-stressful N environment, respectively. These N levels and sampling times would also provide sufficient plant material for analysis.

IV.6. References

Allen E J and Morgan D G 1972 A quantitative analysis of the effects of nitrogen on the growth, development and yield of oilseed rape. J. of Agric. Sci., Camb. 78, 315-324.

Allen E J and Morgan D G 1975 A quantitative comparison of growth and development and yield of different varieties of oilseed rape. J. of Agric. Sci., Camb. 85, 159-174.

Allen E J, Morgan D G and Ridgman W J 1971 A physiological analysis of the growth of the oilseed rape. J. of Agric. Sci., Camb. 77, 339-341.

Baker R J 1994 Breeding methods and selection indices for improved tolerance to biotic and abiotic stresses in cool season food legumes. Euphytica 73, 67-72.

Caradus J R and Dunlop J 1979 Screening white clover plants for efficient phosphorus use. *In* Plant Nutrition 1978. Proc 8th Int. Colloq. on Plant Analysis and Fertilizer Problems. Eds. A R Ferguson, R L Bieleski and I S Ferguson pp 75-82. DSIR Inf. Ser. No. 134. Government Printer, Wellington, New Zealand.

Clark R B 1990 Physiology of cereals for mineral nutrient uptake, use, and efficiency. *In* Crops as enhancers of nutrient use. Eds. B C Baligar and R R Duncan pp 131-209. Academic Press, San Diego, USA.

Clark R B and Duncan R R 1991 Improvement of plant mineral nutrition through breeding. Field Crops Res. 27, 219-240.

Cromer R N, Kriedemann P E, Sands P J and Steweart L G 1993 Leaf growth and photosynthetic response to nitrogen and phosphorus in seedling trees of *Gmelina arborea*. Aust. J. of Plant Physiol. 20, 83-98.

Devine T E 1982 Genetic fitting of crops to problem soils. *In* Breeding plants for less favorable environments. Eds. M N Christiansen and C F Lewis pp 143-173. John Wiley & Sons, New York, USA.

Devine T E, Bouton J H and Mabrahtu T 1990 Legume genetics and breeding for stress tolerance and nutrient efficiency. *In* Crops as enhancers of nutrient use. Eds. B C Baligar and R R Duncan pp 211-252. Academic Press, San Diego, USA.

Duncan R R and Baligar V C 1990 Genetics, breeding, and physiological mechanisms of nutrient uptake and use efficiency: An overview. *In* Crops as enhancers of nutrient use. Eds. B C Baligar and R R Duncan pp 3-35. Academic Press, San Diego, USA. 3-35.

Gabelman W H 1976 Genetic potentials in nitrogen, phosphorus and potassium efficiency. *In* Plant adaptation to mineral stress in problem soils. Ed. M J Wright pp 205-212. Cornell University Press, New York, USA.

Gerath H H and Balko C 1995 The influence of genotype and donor plant on the nitrogen efficiency of DH-lines of winter rape. *In* Proc. GCIRC 9th Int. Rapeseed Congr.: Rapeseed today and tomorrow. Cambridge, UK. Vol. 3, 813-815.

Gerath H H and Schweiger W 1991 Improvement of the use of nutrients in winter rape. A strategy of economically and ecologically responsible fertilizing. *In* Proc. GCIRC 8th Int. Rapeseed Congr. Saskatoon, Canada. Vol. 4, 1197-1201.

Gerloff G C 1963 Comparative mineral nutrition in plants. Ann. Rev. Plant Physiol. 14, 699-704.

Glass A D M 1990 Ion absorption and utilization: The cellular level. *In* Crops as enhancers of nutrient use. Eds. B C Baligar and R R Duncan pp 37-64. Academic Press, San Diego, USA.

Gomez K A and Gomez A A 1984 Statistical procedures for agricultural research. John Wiley & Sons, New York, USA. 680 p.

Grime J P 1989 Whole-plant responses to stress in natural and agricultural systems. *In* Plants under stress: Biochemistry, physiology and ecology and their application to plant improvement. Eds. H G Jones, T J Flowers and M B Jones pp 31-46. Cambridge University Press, Cambridge, UK.

Harper F R 1973 A key to standardize the description of growth stages in turnip rape, *Brassica campestris*. Can. Plant Dis. Surv. 53, 93-95.

Holmes M R J 1980 Nutrition of the oilseed rape crop. Applied Science Publishers Ltd., London, UK. 158 p.

MacAdam J W, Volenec J J and Nelson C J 1989 Effects of nitrogen on mesophyll cell-division and epidermal-cell elongation in tall fescue leaf blades. Plant Physiol. 89, 549-556.

Marschener H 1995 Mineral nutrition of higher plants. 2nd ed. Academic Press, London, UK. 889 p.

McDonald A J S 1989 Nitrate availability and shoot area development in small willow (*Salix viminalis*). Plant Cell and Environ. 7, 199-206.

Mendham N J and Scott R K 1975 The limiting effect of plant size at inflorescence initiation on subsequent growth and yield of oilseed rape (*Brassica napus*). J. of Agric. Sci., Camb. 84, 487-502.

Ogunlela V B, Kullmann A and Geisler G 1989 Leaf growth and chlorophyll content of oilseed rape (Brassica napus L.) as influenced by nitrogen supply. J. Agron. Crop Sci. 163, 73-89.

Ogunlela V B, Kullmann A and Geisler G 1990 Nitrogen distribution and dry matter accumulation in oilseed rape (*Brassica napus* L.) as influenced by N nutrition. J. Agron. and Crop Sci. 164, 321-333.

Richards F J 1959 A flexible growth function for empirical use. J. Exp. Bot. 10, 290-300.

Richards F J 1969 The quantitative analysis of growth. *In* Plant Physiology: A Treatise, Vol. VA: Analysis of Growth: Behavior of Plants and their Organs. Ed. F C Steward pp 3-76. Academic Press, New York, USA.

Rood S B and Major D J 1984 Influence of plant density, nitrogen, water supply and pod or leaf removal on growth of oilseed rape. Field Crops Res. 8, 323-331.

SAS Institute 1991 SAS System for Regression. 2nd ed. SAS Institute, Cary, USA.

SAS Institute 1992 SAS/STAT User's Guide. Version 6. 4th ed. SAS Institute, Cary, USA.

Scott R K, Ogunremi E A, Ivins J D and Mendham N J 1973 The effect of sowing date and season on growth and yield of oilseed rape (*Brassica napus*). J. Agric. Sci., Camb. 81, 277-285.

Siddiqi M Y and Glass A D M 1981 Utilization index: a modified approach to the estimation and comparison of nutrient utilization efficiency in plants. J. Plant Nutr.4, 289-302.

Smith P L 1979 Splines as a useful and convenient statistical tool. Amer. Stat. 33, 57-62.

Stoskopf N C 1981 Understanding crop production. Reston Publishing Company, Reston, USA. 433 p.

Swokowski E W 1979 Calculus with analytical geometry. Prindle, Weber & Schmidt, Boston, USA. 999 p.

Taylor G J and Foy C D 1985 Mechanisms of aluminum tolerance in *Triticum aestivum* (wheat). IV. The role of ammonium and nitrate nutrition. Can. J. Bot. 63, 2181-2186.

Thurling N 1974 Morphophysiological determinants of yield in rapeseed (*Brassica campestris* and *Brassica napus*): I. Growth and morphological characters. Aust. J. Agric. Res. 25, 697-710.

Thurling N 1993 Physiological constraints and their genetic manipulation. *In* Breeding oilseed brassicas. Eds. K S Labana, S S Banga and K S Banga pp 44-66. Springer-Verlag, Berlin, Germany.

Yau S K and Thurling N 1986 Variation in nitrogen response among spring rape (*Brassica napus*) cultivars and its relationship to nitrogen uptake and utilization. Field Crops Res. 16, 139-155.

Vose P B 1963 Varietal differences in plant nutrition. Herbage Abstr. 33, 1-13.

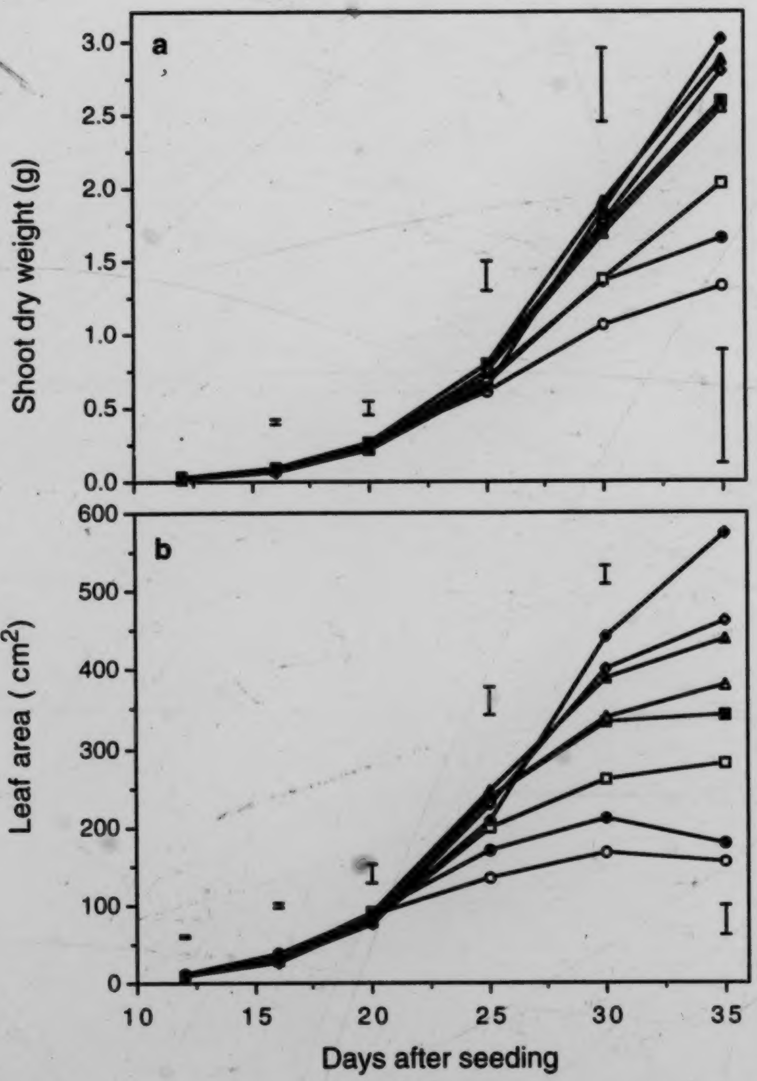


Fig. IV.1. The effect of N supply (○ 0.0; ● 0.6; □ 1.5; ■ 2.4; △ 3.3; ▲ 4.2; ◇ 5.1; ◆ 8.0; mmol N) on the time course of shoot dry weight accumulation (a) and leaf area increase (b) of *B. napus* cv. Alto. Vertical bars at each harvest represents LSD ($p \leq 0.05$) for comparisons between N-treatment means.

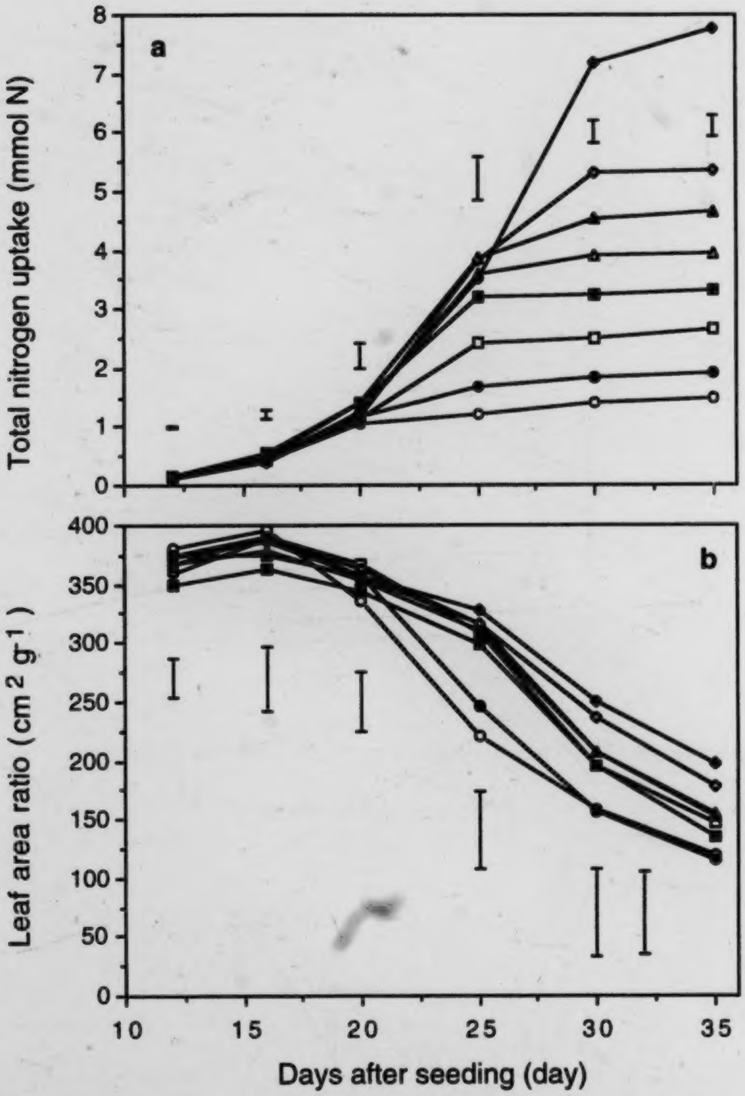


Fig. IV.2. The effect of N supply (○ 0.0; ● 0.6; □ 1.5; ■ 2.4; △ 3.3; ▲ 4.2; ◇ 5.1; ◆ 8.0; mmol N) on the time course of total nitrogen uptake (a) and leaf area ratio (b) of *B. napus* cv. Alto. Vertical bars at each harvest represents LSD ($p \leq 0.05$) for comparisons between N-treatment means.

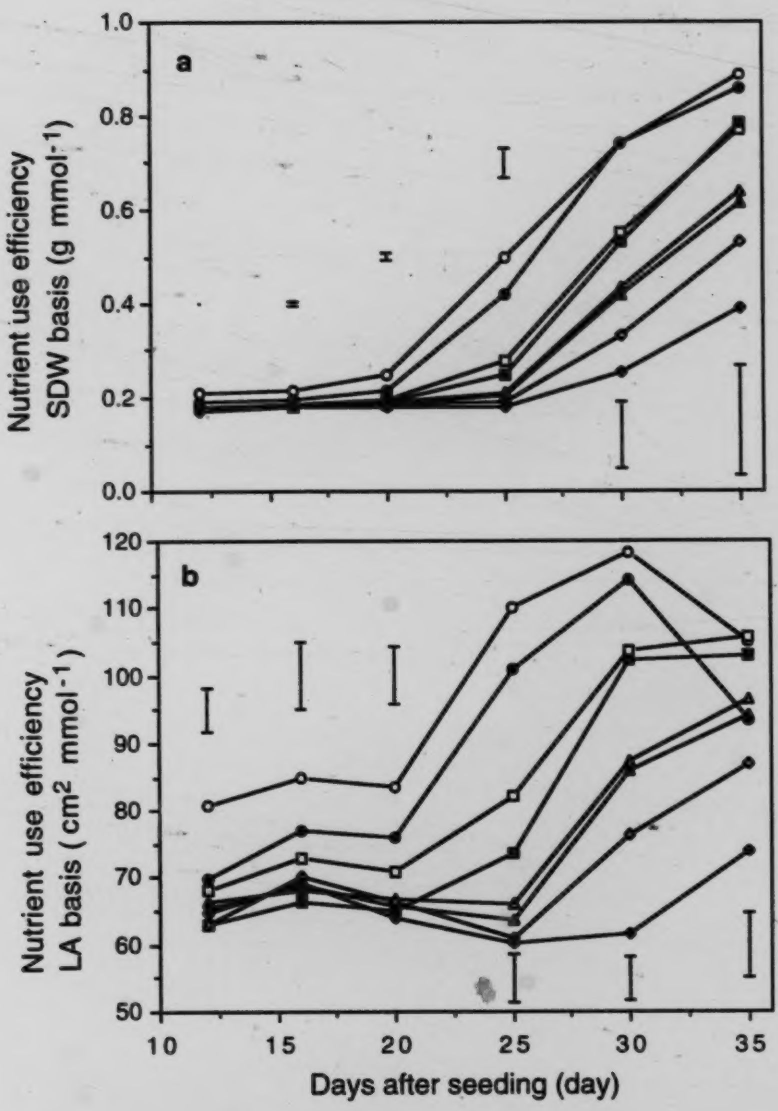


Fig. IV.3. The effect of N supply (○ 0.0; ● 0.6; □ 1.5; ■ 2.4; △ 3.3; ▲ 4.2; ◇ 5.1; ◆ 8.0; mmol N) on the time course of nitrogen use efficiency on a shoot dry weight basis (a) and leaf area basis (b) of *B. napus* cv. Alto. Vertical bars at each harvest represents LSD ($p \leq 0.05$) for comparisons between N-treatment means.

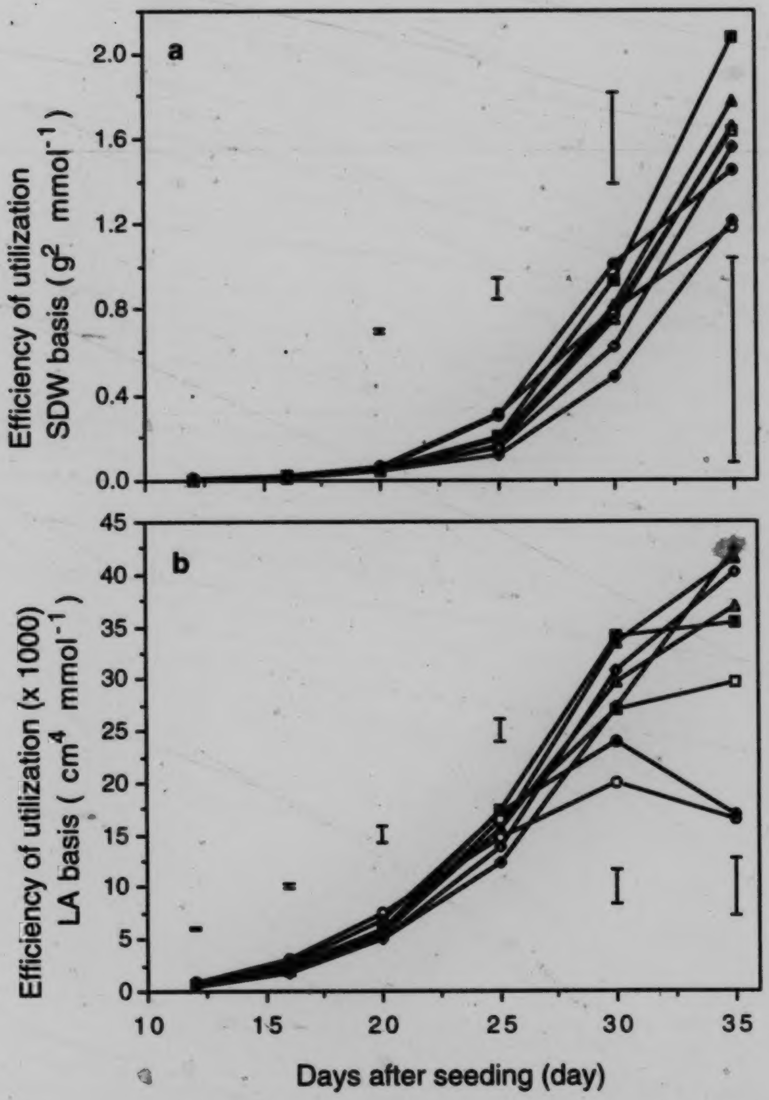


Fig. IV.4. The effect of N supply (○ 0.0; ● 0.6; □ 1.5; ■ 2.4; △ 3.3; ▲ 4.2; ◇ 5.1; ◆ 8.0; mmol N) on the time course of efficiency of utilization on a shoot dry weight basis (a) and leaf area basis (b) of *B. napus* cv. Alto. Vertical bars at each harvest represents LSD ($p \leq 0.05$) for comparisons between N-treatment means.

Table IV.1. The effect of nitrogen supply on the number of days after seeding (DAS) that LA and TOTN curves underwent their point of inflexion (DAS_{PI}) and the DAS for the Knot point of NUE_{SDW}.

Nitrogen treatment	Point of inflexion (DAS_{PI})		Knot	
(mmol pot^{-1})	LA	TOTN	NUE_{SDW}	[a]$LSD_{0.05}$
0.0	19.5	16.7	18.6	1.4
0.6	21.3	18.8	19.8	2.6
1.5	22.7	21.5	23.3	2.5
2.4	24.1	22.3	24.6	2.5
3.3	23.7	23.5	25.2	2.3
4.2	24.9	22.6	25.9	2.8
5.1	25.5	24.5	26.1	2.5
8.0	28.0	27.4	27.5	3.0
[b]$LSD_{0.05}$	1.8	2.2	2.8	

[c]Regression constants of DAS_{PI} and Knot on nitrogen treatments

a	20.7	18.5	20.5
b	0.96	1.21	1.07
r	0.92***	0.91***	0.85***

[a] = $LSD_{0.05}$ statistics for comparisons between DAS_{PI} means within each N-treatment.

[b] = $LSD_{0.05}$ statistics for comparisons within DAS_{PI} means between each N-treatment.

[c] = Regression constants shown for linear regressions of the form: $DAS_{PI} = a+bN$, where DAS_{PI} is days to inflexion point (days) and N is nitrogen treatment (mmol).

*** Significant at the 0.001 probability level.

CHAPTER V

The effect of nitrogen on growth and nitrogen efficiency components in rapeseed (*Brassica napus* L.) late in the rosette stage.

V.1. Introduction

The use of crop cultivars that are more efficient in the uptake and utilization of nitrogen (N) can be an effective way of reducing N pollution and crop inputs (*eg*. Gerloff, 1963, 1976; Vose, 1963; Gabelman, 1976; Clark and Duncan, 1991). Selection for genotypic variation for N-uptake and N-utilization in an important high N-requiring crop such as canola (*Brassica napus* L.) might be exploited for improving the N-efficiency of new cultivars. However, research on the improvement of N efficiency in *B. napus* has been limited (*eg*. Yau and Thurling, 1987; Gerath and Schweiger, 1991; Gerath and Balko, 1995).

A reliable screening technique is necessary to select plant genetic variation for nutrient efficiency (Devine, 1982; Devine *et al.*, 1990). With the objective of developing a technique to screen N-efficient *B. napus*, several factors have been examined. An optimum $NH_4^+:NO_3^-$ ratio for maximum plant growth has been determined (Chapter II). Also, the feasibility of using shoots rather than roots to assess N efficiency has been demonstrated (Chapter III). The later part of the rosette stage has been established as an appropriate sampling time and the possibility of using leaf area expansion and shoot biomass as diagnostic growth components when estimating N efficiency has been demonstrated (Chapter IV). Among other factors requiring examination are the N levels at which to assay *B. napus* genotypes. Selection of a set of N-treatments to assess maximum genetic potential and to assess stress tolerance (Baker, 1994) can be deduced from an N-dose response curve (Berry and Wallace, 1981; Duncan and Baligar, 1990).

Definition of N efficiency for screening germplasm may be classified in two groups (Chapter I). The first group describes the response of growth components relative to the N supplied or absorbed at a particular static N level. This group includes efficiency definitions such as total N absorbed, nitrogen use efficiency, and efficiency of utilization (Clark, 1990; Siddiqi and Glass, 1981). These are static measures of efficiency. The second group of N efficiencies describes the response of growth components to increasing supply per unit of N or absorption of N and include terms such as apparent recovery, agronomic efficiency, and physiological efficiency (Craswell and Godwin, 1984). These measurements express a response to incremental levels of N application and thus are considered to be response rates per unit of N increment. The N levels for which these efficiencies are estimated define the nature of the selection. How these N efficiency components behave in response to N supply in *B. napus* has not been previously determined.

In the present work, the response of *B. napus* cv. Alto to increasing N supply and the resulting effects on plant growth and N-efficiency components were examined. This information would form the basis for the selection of N treatments and N-efficiency components necessary for a screening protocol to select N-efficient *B. napus* germplasm.

V.2. Materials and Methods

V.2.a. Plant materials and growing conditions

Plants of rapeseed (*Brassica napus* L.) cv. Alto were grown in the greenhouse in 10×10 cm square plastic pots filled with a soil-free medium (Vermiculite + Peat Moss + Sand; 3:3:1 volume basis). Each pot was filled with 257 ± 4 g of medium, (dry weight basis), containing 21.1 ± 0.3 mmol N per pot. To prevent nutrient loss, the drainage holes of the pots were sealed with tape. Two to four seeds were planted per pot, and thinned to 1 seedling, 7 days after seeding (DAS). Seeds of cv. Alto contained an average of 9.98 mmol N per 1000 seeds.

A modified basal nutrient solution (Taylor and Foy, 1985) was added to each individual pot providing (mmol): K, 1.60; PO_4, 0.40; SO_4, 0.40; Mg, 0.60; Cl, 1.34; Mn, 8×10^{-3}; B, 2.4×10^{-2}; Zn, 2×10^{-3}; Cu, 6×10^{-4}; Na, 8.1×10^{-2}; Mo, 4×10^{-4}; EDTA, 0.04; Fe, 0.04. Iron was supplied as Fe-EDTA prepared from equimolar amounts of $FeCl_3$ and Na_2EDTA. All nutrients were supplied in solution, except for Ca which was supplied as powdered $CaSO_4$ and mixed into the medium. A nitrogen (N) solution consisting of 90% $N\text{-}NO_3^-$ and 10% $N\text{-}NH_4^+$ provided 20 N-treatments, from 0 to 8.0 mmol, corresponding to a range of 0 to 112 kg N ha^{-1}. An earlier pot experiment demonstrated the positive response of *B. napus* to N supply when $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ were supplied in a mixture rather than in isolation (Chapter II). Measured volumes of all nutrients, including N-treatments, were added to each pot 2 DAS. Plants were watered regularly with distilled water and care was taken to prevent waterlogging.

Plants were grown under greenhouse conditions at a temperature of 20-23 °C and relative humidity of 60-90%. The photosynthetic photon flux density (PPFD) was 1303 ± 38 µmol m^{-2} s^{-1} when days were sunny and clear. During cloudy days, natural light was supplemented with 400 watt, high pressure sodium lamps (Sylvania) attached to high intensity discharge fixtures providing a basal average PPFD of 292 ± 17 µmol m^{-2} s^{-1} for 16 h daily at soil surface height.

V.2.b. Plant growth and N efficiency components measured

Shoots were harvested individually, cut at the base of the cotyledons, 28 DAS when most plants were in the rosette stage (Harper, 1973). Roots were not harvested since an earlier study indicated that shoots and not roots were the principal plant components accounting for differences in N efficiency (Chapter III).

Measured variables included leaf area (LA), shoot fresh weight (SFW), number of leaves per plant (LNUM), shoot dry weight (SDW), and percent N content in the shoot tissue (N%). Leaf area was measured with a LI-COR LI3100 Area Meter (LI-COR Inc., Lincoln, Nebraska, USA). Leaf presence on a plant was recorded if the leaf lamina was longer than 0.5 cm. Samples were dried in a forced-air oven to constant weight at 65 C°, weighed, and ground in a Wiley mill fitted with a 40 mesh size screen (Arthur H. Thomas Co., Scientific Apparatus, Philadelphia, PA, USA). Percent nitrogen was measured by the combustion method using a LECO FP-2000 Nitrogen-Protein analyzer (LECO Corporation, St. Joseph, MI, USA). A homogeneous subsample of approximately 0.7-0.9 g was used to determine N%.

Calculated parameters included leaf area ratio (LA/SDW = LAR), plant density (SFW/SDW = DENSITY) and total N absorbed

(TOTN = $((((\mathrm{SDW} \times \mathrm{N\%})/100)/14.0) \times 1000))$ (mmol^{-1} N). The parameters used to characterize the efficiency of N acquisition and use by cv. Alto, and the efficiency of response to increasing N supply were calculated using the following equations:

Equation 1: Nitrogen use efficiency (NUE) = SDW/TOTN (g mmol^{-1} N)

Equation 2: Efficiency of utilization (EU) = SDW×(SDW/TOTN) (g^{-2} mmol^{-1} N)

Equation 3: Physiological efficiency (PE) = $(\mathrm{SDW}_{\mathrm{High\ N}} - \mathrm{SDW}_{\mathrm{Low\ N}})/(\mathrm{TOTN}_{\mathrm{High\ N}} - \mathrm{TOTN}_{\mathrm{Low\ N}})$ (g mmol^{-1} N)

Equation 4: Agronomic efficiency (AE) = $(\mathrm{SDW}_{\mathrm{High\ N}} - \mathrm{SDW}_{\mathrm{Low\ N}})$/net N applied) (g mmol^{-1} N)

Equation 5: Apparent recovery (AR) = $((\mathrm{TOTN}_{\mathrm{High\ N}} - \mathrm{TOTN}_{\mathrm{Low\ N}})$/net N applied)) × 100 (%)

Where: Low N = N-treatment taken as control N-treatment; High N = N-treatment higher than control; net N applied = difference between High N and Low N. Equation 2 was taken from Siddiqi and Glass (1981) whereas equations 3, 4 and 5 were taken from Craswell and Godwin (1984). Efficiency components on a LA basis were obtained by replacing the SDW term with LA in the equations,

V.2.c. Experimental design and statistical analysis

The experimental design was a randomized complete block, consisting of 20 N-treatments, six plants per N-treatment and five replications for a total of 600 pots. Replications were staggered over time and harvested accordingly. Polynomials to the 3rd power were fitted to the means of the response curves of each measured parameter. Each data point represented the value of 30 observations. These polynomial equations described the overall trend and shape of the response curves and were used in calculating the N-efficiency parameters AE, AR and PE.

Values of AE, AR and PE were calculated by systematically selecting a combination of both a Low N and a High N (see equations 3, 4 and 5). These two N-treatments were then used to calculate the SDW and TOTN from the polynomial equations derived from the empirical data (Fig. V.1, and Fig. V.3a, respectively). The difference between the two N-treatments was used as net N applied. Both N-treatments chosen, Low N and High N, and the values calculated from the polynomials for SDW and TOTN were then used to calculate the efficiency parameters (ie. AE, AR and PE) and each resulting value was plotted against the N-treatment chosen as High N. The N-treatments selected as Low N were 0.0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mmol N while, the High N was selected incrementally at small intervals of 0.02 mmol N from 0.01 to 8.0 mmol N. For each efficiency parameter seven curves were developed. Each of the seven curves for each efficiency parameter represents an N-treatment selected as a Low N while the shape of each curve reflects the N-treatment incrementally selected as High N. All calculations and graphs were developed using the modeling software package STELLA II (STELLA II, High Performance Systems Inc., Hanover, NH, USA).

V.3. Results

V.3.a. General responses to N supply

The majority of plants were between the end of the rosette stage and the beginning of stem elongation stage at sampling time, regardless of the N-treatment (Harper, 1973). Plants grown in treatments with less than 1.0 mmol N exhibited N deficiency symptoms (*eg*. leaf chlorosis; Salisbury and Ross, 1992; Marschener, 1995), however, no leaf shedding was observed. Each pot contained an average of 21.1 mmol N before N addition, while seeds contained an average background level of 9.98×10^{-3} mmol N per seed. An estimated 0.7 mmol N was absorbed by the shoots in the 0 N-treatment (Fig. V.3a). This indicated that most of the native N in the medium was not available for growth. The combined seed and media levels of N, therefore, were not large enough to significantly influence the effects of increasing N supply on plant growth (Fig. V.1, V.2).

V.3.b. Response of plant growth components to N supply

Shoot dry weight and LA increased in response to high N supply in a hyperbolic fashion (Fig. V.1). Increasing N-supply resulted in a SDW increase by a factor of 3 and a LA increase by a factor of 4 (Fig. V.1), with maxima obtained 3 to 5 mmol N (Fig. V.1). Increased N supply also resulted in increased LNUM, DENSITY and LAR (Fig. V.2a-c). A maximum of about 6-7 leaves per plant were produced above 1.5 mmol N supply (Fig. 2a). Thus, leaf number production was not responsive to increasing N supply and appeared to be a determinate trait. Leaf area ratio increased from 200 $cm^2\ g^{-1}$ to 280 $cm^2\ g^{-1}$ as the supply of N increased (Fig. V.2b). This indicated that Alto 'partitioned' more resources into LA rather than SDW and plants became more 'leafy'. This greater 'leafiness', however, was not the result of an increased number of leaves. Plant density decreased with increasing N supply. From a level of 10 g SFW g^{-1} SDW, at the lowest N treatment, a steady increase to a maximum of 14 g SFW g^{-1} SDW at the highest N-treatment. This indicated an increase in shoot succulence as the N level increased (Fig. V.2c).

Total nitrogen absorption (TOTN) increased linearly up to about 4.0 mmol N-treatment followed by a curvilinear response up to the highest N-treatment (Fig. V.3a). Above 3.0 mmol of N-treatment, the total N recovered was less than the N supplied at the beginning of the experiment. The linear response of TOTN up to about 3 mmol N indicated that Alto had absorbed most of the available N in the growing medium, whereas above 3 mmol N, there might still have been N available for growth (Fig. V.3a). The increased N absorption resulted in SDW and LA increasing in a curvilinear fashion (Fig. V.3b). Relatively similar levels of growth response of SDW and LA were obtained above 3.0 and 4.0 mmol of absorbed N, respectively (Fig. V.3b). Thus, critical N concentrations for maximum response of SDW (Fig. V.1) growth and LA (Fig. V.3b) expansion were not the same.

V.3.c. Estimates of static nitrogen efficiency components

Nitrogen use efficiency (NUE; Fig. V.4a) describes plant yield per unit nitrogen in the tissue and thus is the reciprocal of nitrogen concentration (Glass, 1990). Nitrogen use efficiency either on a SDW basis (NUE_{SDW}) or a LA basis (NUE_{LA}) decreased curvilinearly as N supply increased resulting in a three- and two-fold reduction, respectively (Fig. V.4a). The higher values obtained at lower N treatment concentration (Fig. V.4a) represented a dilution of N as SDW growth or LA expansion continued to increase (Fig. IV.1) after N becomes unavailable to plants in those same N treatments (Fig. V.3a, V.4a).

Efficiency of utilization (EU) is the product of total yield times NUE and emphasizes the contribution of total plant yield (Glass, 1990; Siddiqi and Glass, 1981). Efficiency of utilization was calculated on a SDW basis (EU_{SDW}) and on a LA basis (EU_{LA}; Fig. V.4b). Maxima EU_{SDW} and EU_{LA} were produced at about 2.0-3.0 and about 3.0-4.0 mmol N, respectively (Fig. V.4b). These maxima levels did not coincide with either the maxima SDW or LA levels (Fig. V.1) nor with the maximum NUE levels (Fig. V.4a). The highest EU_{SDW} responses were obtained in the lower N levels while the lowest EU_{SDW} responses were obtained at both low and high N levels. The responses of EU_{LA}, on the other hand, were observed at the high N levels. The EU increase was the result of the greater rate of increase of SDW or LA relative to the rate of N absorption. The constant EU was a result of the similar rates of increase of SDW and LA relative to the rate of N absorption, while the EU decline occurred when the SDW or LA rate of response to N supply was nil or lower than the N uptake rate.

V.3.d. Estimates of response rate of N efficiency components

Although not shown, AE and PE on a LA basis showed similar response curves as when calculated on a SDW basis (Fig. V.5a-c). Apparent recovery of N is the total N recovered (TOTN) at the High N treatment relative to the total N recovered (TOTN) at the Low N treatment. (Craswell and Godwin, 1984). It is a measure of how efficient a plant is in recovering N fertilizer added to the growing medium (Fig. V.3a). A wide variation of AR estimates were obtained ranging from 0.0 to 81.0% (Fig. V.5a). A relatively constant AR estimate of about 80% between 0-4 mmol High-N was obtained when either 0.0, 1.0 or 2.0 mmol N was used as a Low-N (Fig. V.5a). Selection of both Low-N and High-N below 3 mmol N supply gave the highest and relatively similar AR estimates. This is a reflection of the straight portion of the N-dose response on N absorption (Fig. V.3a). However, the largest plant growth responses were obtained above these N treatments (Fig. V.1). Thus, higher N recovery rates did not indicate higher absolute plant growth.

Agronomic efficiency is the slope of the SDW curve as N supply increases (Fig. V.1). It is the response rate of SDW to increased N supply (Craswell and Godwin, 1984). The estimated AE values were not constant and were the direct result of the N-treatments selected as Low-N and High-N (Fig. V.5b). The largest AE estimates (Fig. V.5b) occurred when SDW response rates to increasing N were the largest and this occurred in the N deficient range (Fig. V.1).

Physiological efficiency is the slope of SDW in response to the total N uptake (Fig. V.3b). It is the response of SDW per unit of N absorbed by the plant (Craswell and Godwin, 1984). Physiological efficiency estimates did not show a constant level regardless of the N-treatments selected as either Low N or High N (Fig. 5C). The highest estimated PE was obtained in N deficiency levels, as was the case for AE estimates. That is, the rate of response of SDW to increasing N declined as the N supply increased (Fig. V.3b).

V.4. Discussion

The levels of the N treatments used in screening N-efficient *B. napus* germplasm define the nature of the genetic selection. If the N level is in the deficient range, N stress will be imposed on the growing plants, otherwise, N stress will be absent. In an N-stressed environment, genotypic tolerance to N deficiency will be assessed while in the non-stressed N environment maximum growth potential will be assessed (Baker, 1994). Under field conditions, a wide variation of N availability exists as a result of the wide range of soil conditions and farming practices. Therefore, *B. napus* genotypes are desirable which are able to have a relatively high yield not only at Low or High N, but are also responsive to increasing N supply.

Crop genotypes can respond differentially to increased N supply (*eg*. Vose, 1990; Sattelmacher *et al*., 1994). Genotypic selection under suboptimal N levels may not guarantee that selected genotypes will respond to incrementally higher N supply, nor will they necessarily show the largest growth potential under normal conditions (*eg*. Muruli and Paulsen, 1981). There is thus a lack of consensus regarding selection criteria. Some have suggested that genotypic selection should be done under suboptimal N levels (Evans, 1991), while others have recommended intermediate N fertility levels (Balko and Russell, 1980) or extreme or modest N levels (Muruli and Paulsen, 1981). From this study, several Low and High N-treatment combinations could be used for assessing both static (NUE and EU) and response rate (AR, AE and PE) N efficiencies in *B. napus* (Fig. V.4ab, V.5a-c). Furthermore, the response curves of the estimated efficiencies provide clear evidence of the relative nature of these estimates (Fig. V.4ab, V.5a-c). In the context of this assay, selection of N treatments for a two N-levels screening protocol could be at or below 1.5 mmol N, and at or above 5.1 mmol N in order to provide both stress and non-stress N environments. A 1.5 mmol N treatment produced approximately 50% growth response, while a 5.1 mmol N treatment produced approximately 100% growth response (Fig. V.1).

Suitable growth parameters for estimating N-efficiency should be sensitive to N deficiency and be related to the final economic yield (Devine et al., 1990). A large number of growth parameters have been used in estimating N-efficiency in crop plants (see references in Clark, 1990). I have already established that the latter part of the rosette stage is an appropriate sampling time for screening N-efficient *B. napus* germplasm (Chapter IV). Several growth parameters at this stage are potentially useful for screening. In the present study LA was more responsive than SDW to N supply and appears, along with SDW, to be suitable for ranking genotypes for N efficiency (Fig.

V.1). Leaf expansion of *B. napus* has been shown to be responsive to N supply (Chapter IV; Ogunlela *et al.*, 1990; Rood and Major, 1984; Allen *et al.*, 1971) and its sensitivity to N-deficiency appears to be a potentially useful diagnostic character for N stress. The other growth components (LNUM, LAR and DENSITY) were less sensitive to increasing N supply (Fig. V.2) and are therefore less suitable for screening *B. napus* germplasm. However, the final selection of the growth components suitable for screening will depend on the available genotypic variability in the *B. napus* population.

The three efficiency components AR, AE and PE are interrelated: AE = AR×PE (Craswell and Godwin, 1984). Consequently, AE can be viewed as a reflection of the integrated efficiency with which N supplied is used by the plant (Craswell and Godwin, 1984). An increase in either AR or PE or both will result in an increase of AE (Novoa and Loomis, 1981). In the context of a breeding program, selection for the three response-rate N efficiency estimates would be potentially useful if not necessary. Although some genotypes may have both a high AR and PE it may not occur for all genotypes. Thus, there might be a need to select for each parameter separately amongst genotypes. A recombination program could be attempted to combine the best N-efficiency characteristics into a genotype.

The wide range of Low and High N combinations available for estimating these efficiency components resulted in a wide range of values for each N efficiency component (Fig. V.3b, V.5a-c). This is similar to the NUE and EU response to N supply. Also, high efficiency estimates, either static or response rates, did not measure maximum growth response in sufficient N nor maximum tolerance in N deficiency. Therefore, genotypic characterization and ranking must be based on an assessment of both growth component responses and estimates of both static and rate response efficiencies to N supply.

V.5. Conclusions

Screening *B. napus* genotypes for N-efficiency should be performed in both N stressed and non-stressed conditions. For a 28-day growth period an N-level of 1.5 mmol as Low N, and an N-level of 5.1 mmol as High N, would provide about a 50 and a 100% growth response, respectively, and should induce both stress and non-stress N environments. These N levels will provide N environments in which both static and response rate efficiency estimates can be made.

V.6. References

Allen E J, Morgan D G and Ridgman W J 1971 A physiological analysis of the growth of the oilseed rape. J. Agric. Sci., Camb. 77, 339-341.

Baker R J 1994 Breeding methods and selection indices for improved tolerance to biotic and abiotic stresses in cool season food legumes. Euphytica 73, 67-72.

Balko L G and Russell W A 1980 Effects of rates of nitrogen fertilizer on maize and inbred lines and hybrid progeny. I. prediction of yield response. Maydica 25, 65-79.

Berry W L and Wallace A 1981 Toxicity: The concept and relationship to the dose response curve. J. Plant Nutr. 3, 13-19.

Clark R B 1990 Physiology of cereals for mineral nutrient uptake, use, and efficiency. *In* Crops as enhancers of nutrient use. Eds. B C Baligar and R R Duncan pp 131-209. Academic Press, San Diego, USA.

Clark R B and Duncan R R 1991 Improvement of plant mineral nutrition through breeding. Field Crops Res. 27, 219-240.

Craswell E T and Godwin D C 1984 The efficiency of nitrogen fertilizers applied to cereals in different climates. Adv. Plant Nutr. 1, 1-55.

Devine T E 1982 Genetic fitting of crops to problem soils. *In* Breeding plants for less favorable environments. Eds. M N Christiansen and C F Lewis pp 143-173. John Wiley & Sons, New York, USA.

Devine T E, Bouton J H and Mabrahtu T 1990 Legume genetics and breeding for stress tolerance and nutrient efficiency. *In* Crops as enhancers of nutrient use. Eds. B C Baligar and R R Duncan pp 211-252. Academic Press, San Diego, USA.

Duncan R R and Baligar V C 1990 Genetics, breeding, and physiological mechanisms of nutrient uptake and use efficiency: An overview. *In* Crops as enhancers of nutrient use. Eds. B C Baligar and R R Duncan pp 3-35. Academic Press, San Diego, USA.

Evans A S 1991 Leaf physiological aspects of nitrogen-use efficiency in *Brassica campestris* L.: quantitative variation across nutrient treatments. Theor. Appl.Genet. 81, 64-70.

Gabelman W H 1976 Genetic potentials in nitrogen, phosphorus and potassium efficiency. *In* Plant adaptation to mineral stress in problem soils. Ed. M J Wright pp 205-212. Cornell University Press, New York, USA.

Gerath H H and Balko C 1995 The influence of genotype and donor plant on the nitrogen efficiency of DH-lines of winter rape. *In* Proceedings GCIRC 9th international rapeseed congress: Rapessed today and tomorrow. Cambridge, UK. Vol. 3, pp 813-815.

Gerath H H and Schweiger W 1991 Improvement of the use of nutrients in winter rape. A strategy of economically and ecologically responsible fertilizing. *In* Proceedings GCIRC eight international rapeseed congress. Saskatoon, Canada. Vol 4, 1197-1201.

Gerloff G C 1963 Comparative mineral nutrition in plants. Ann. Rev. Plant Physiol. 14, 699-704.

Gerloff G C 1976 Plant efficiencies in the use of nitrogen, phosphorus and potassium. *In* Plant adaptation to mineral stress in problem soils. Ed. M J Wright pp 161-173. Cornell University Press, New York, USA.

Glass A D M 1990 Ion absorption and utilization: The cellular level. *In* Crops as enhancers of nutrient use. Eds. V C Baligar and R R Duncan pp 37-64. : Academic Press, San Diego, USA.

Harper F R 1973 A key to standardize the description of growth stages in turnip rape, *Brassica campestris*. Can. Plant Dis. Surv. 53, 93-95.

Marschener H 1995 Mineral nutrition of higher plants. 2nd ed. Academic Press, London, UK. 889 p.

Muruli B I and Paulsen G M 1981 Improvement of nitrogen use efficiency and its relationship to other traits in maize. Maydica 26, 63-73.

Novoa R and Loomis R S 1981 Nitrogen and plant production. Plant Soil 58, 177-204.

Ogunlela V B, Kullmann A and Geisler G 1990 Nitrogen distribution and dry matter accumulation in oilseed rape (*Brassica napus* L.) as influenced by N nutrition. J. Agron. Crop Sci. 164, 321-333.

Rood S B and Major D J 1984 Influence of plant density, nitrogen, water supply and pod or leaf removal on growth of oilseed rape. Field Crops Res. 8, 323-331.

Salisbury F B and Ross C W 1992 Plant physiology. 4th ed. Wadsworth Publishing Company, Belmont, USA. 682 p.

Sattelmacher B W, Horst J and Becker H C 1994 Factors that contribute to genetic variation for nutrient efficiency of crop plants. J. Plant Nutr. Soil Sci. 157, 215-224.

Siddiqi M Y and Glass A D M 1981 Utilization index: a modified approach to the estimation and comparison of nutrient utilization efficiency in plants. J. Plant Nutr. 4, 289-302.

Taylor G J and Foy C D 1985 Mechanisms of aluminum tolerance in *Triticum aestivum* (wheat). IV. The role of ammonium and nitrate nutrition. Can. J. Bot. 63, 2181-2186.

Vose P B 1963 Varietal differences in plant nutrition. Herbage Abst. 33, 1-13.

Vose P B 1990 Plant nutrition relationships at the whole plant level. *In* Crops as enhancers of nutrient use. Eds. V C Baligar R R Duncan pp 65-80. Academic Press, San Diego, USA.

Yau S K and Thurling N 1987 Genetic variation in nitrogen uptake and utilization in spring rape (*Brassica napus* L.) and its exploitation through selection. Plant Breed. 98, 330-338.

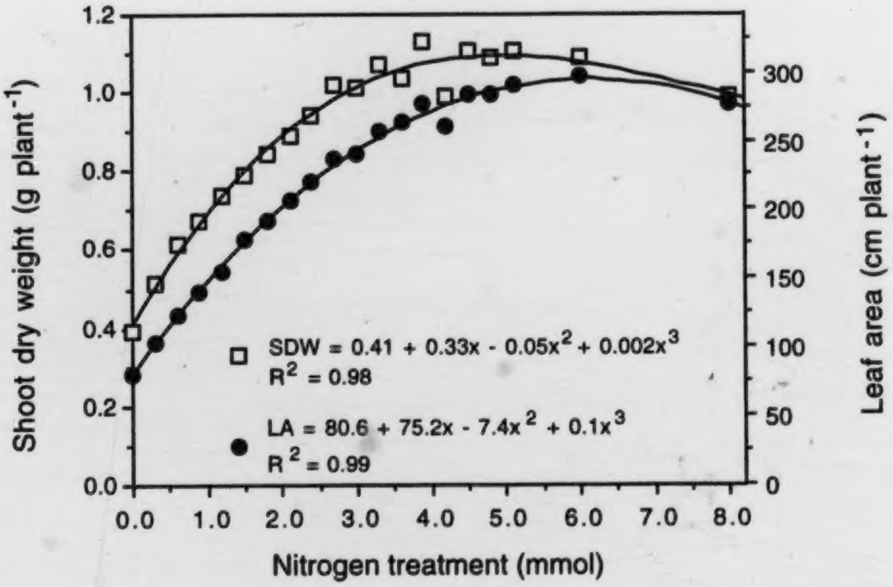


Fig. V.1. The effect of N supply on shoot dry weight (SDW) and leaf area (LA) of *B. napus* cv. Alto plants harvested at 28 days after seeding.

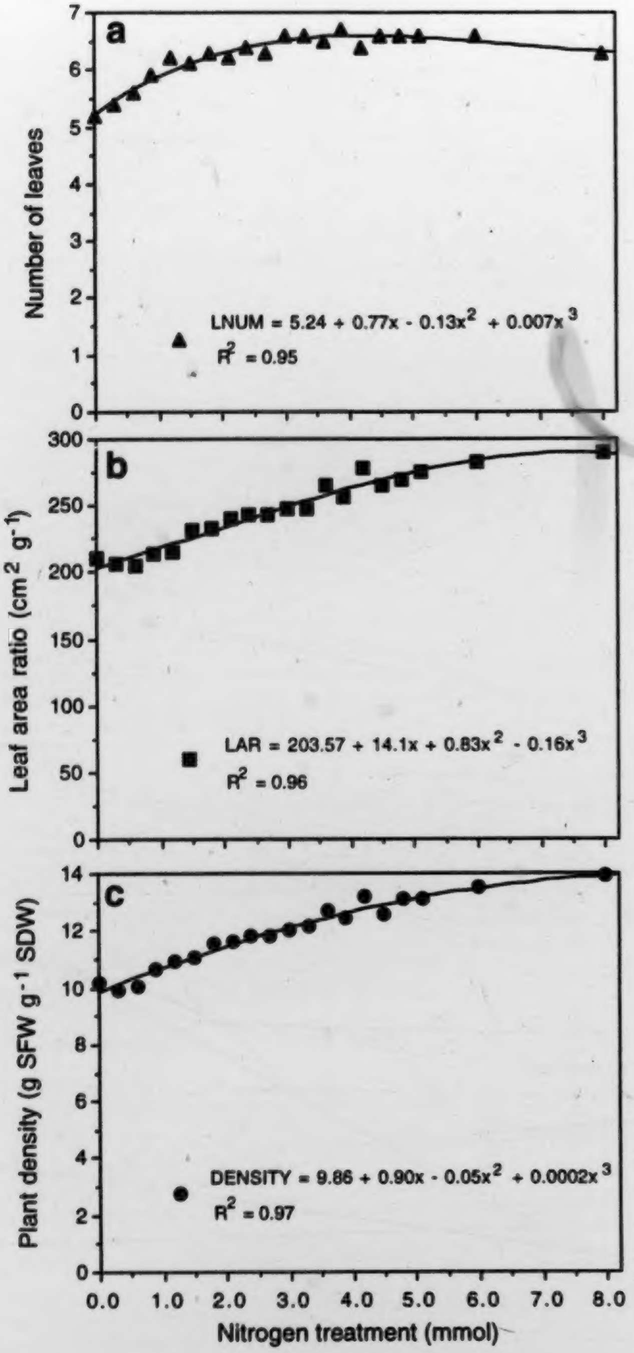


Fig. V.2. The effect of increasing N supply on number of leaves (LNUM; a), leaf area ratio (LAR; b) and plant density (DENSITY; c) of *B. napus* cv. Alto plants harvested at 28 days after seeding.

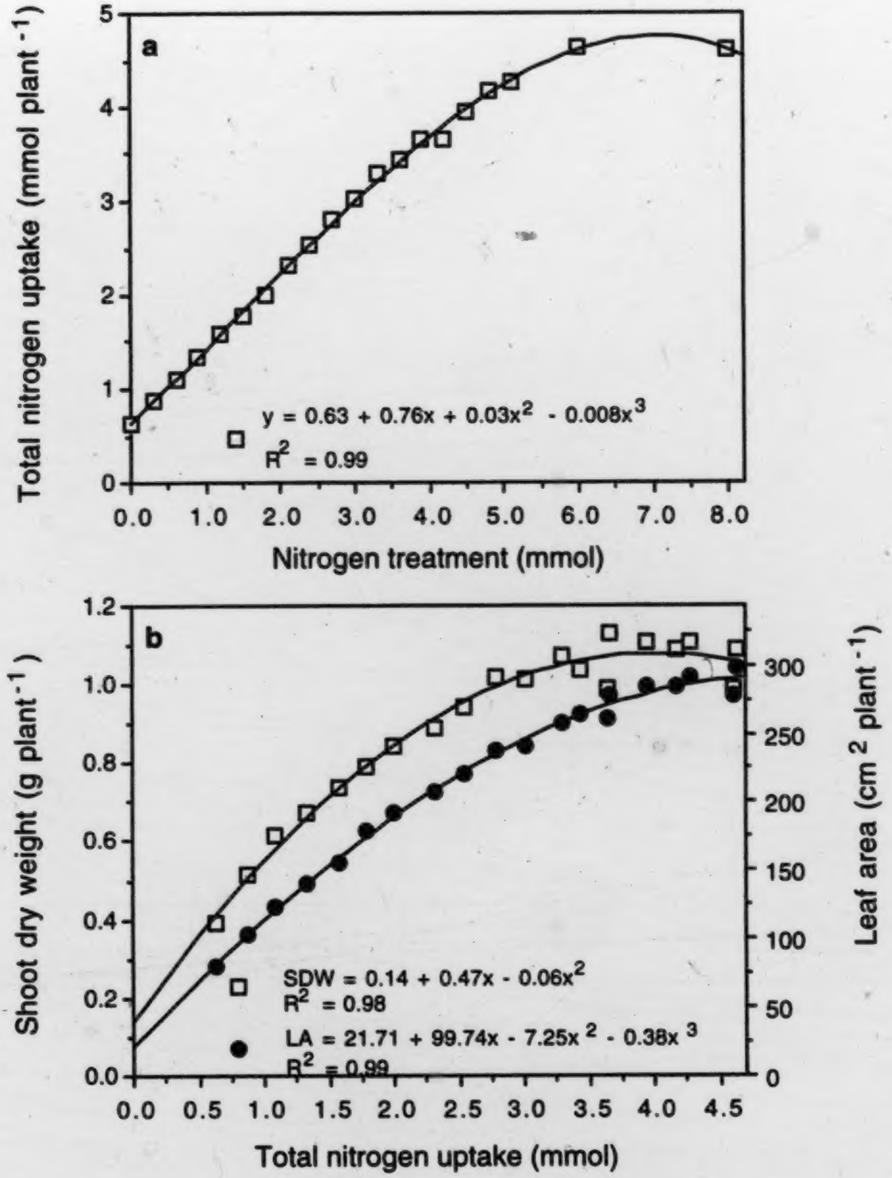


Fig. V.3. The (a) effect N supply on total N uptake (TOTN) and the (b) response of shoot dry weight (SDW) and leaf area (LA) to total N uptake (TOTN) of *B. napus* cv. Alto plants harvested at 28 days after seeding.

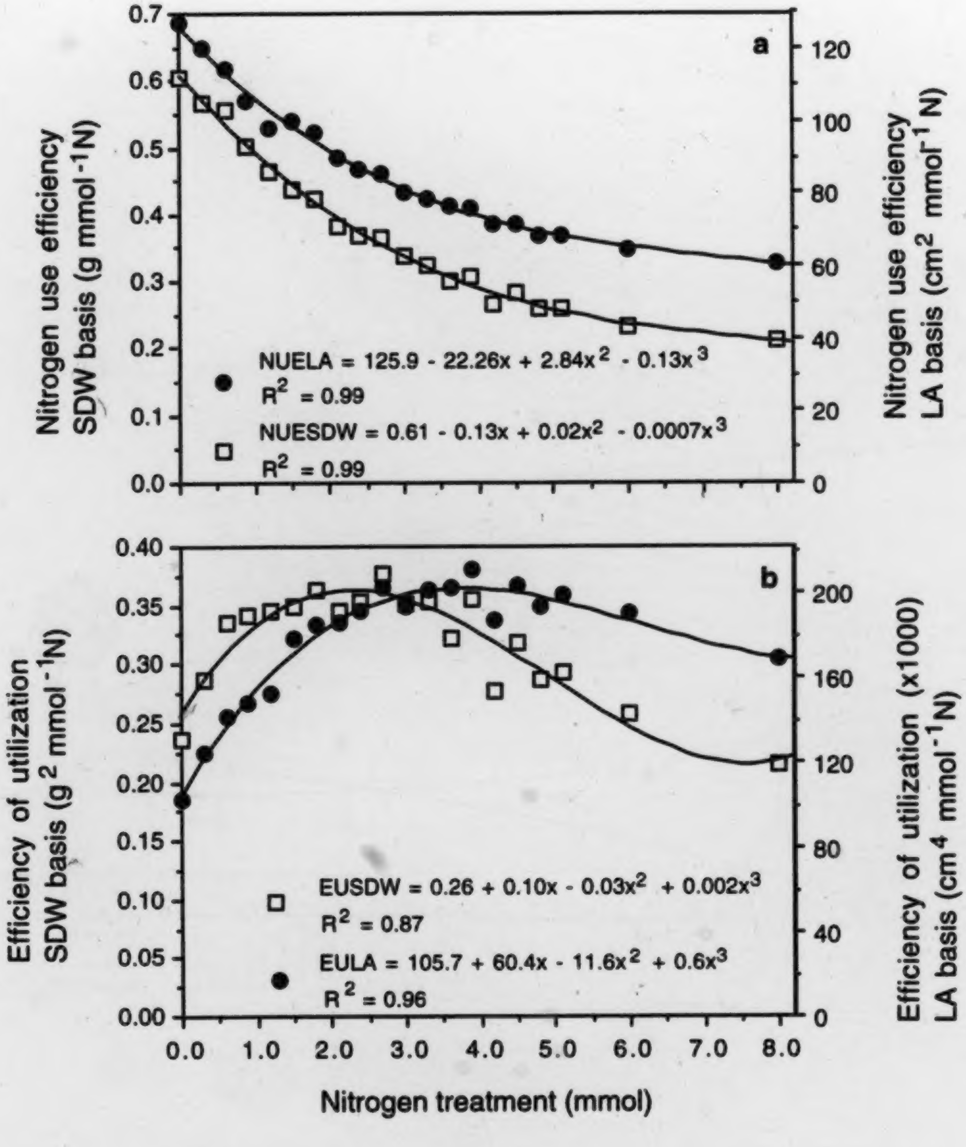


Fig. V.4. The effect of N supply on (a) nitrogen use efficiency (NUE) and (b) on efficiency of utilization (EU), on a SDW and LA basis, of *B. napus* cv. Alto plants harvested at 28 days after seeding.

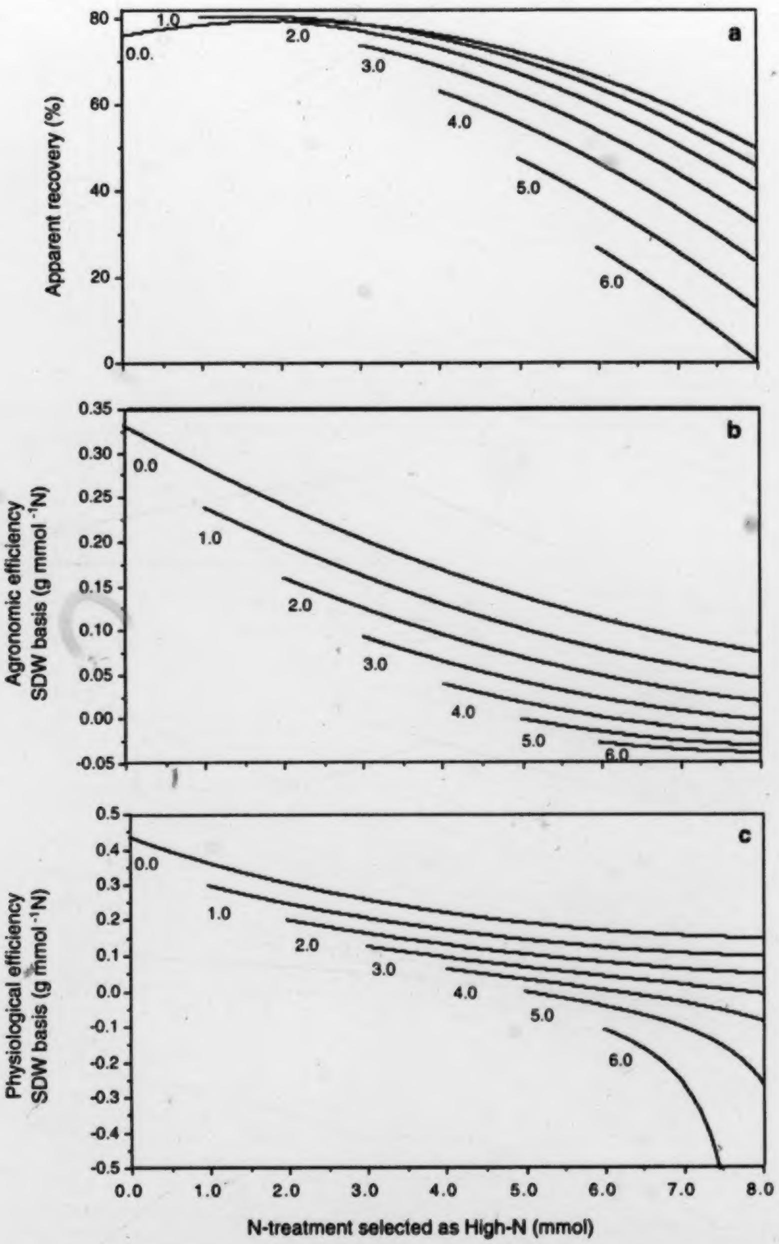


Fig. V.5. Estimates of (a) apparent recovery of N (AR) and (b) agronomic efficiency and (c) physiological efficiency on a shoot dry weight basis of *B. napus* cv. Alto plants harvested at 28 days after seeding. Each curve reflects the N treatments selected as Low-N (0.0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mmol N) while, the shape of each curve reflects the N-treatments selected as High-N.

CHAPTER VI

Variability of nitrogen efficiency components in rapeseed (*Brassica napus* L.) germplasm grown at deficient and sufficient nitrogen levels.

VI.1. Introduction

Genotypic variation for the efficient absorption and utilization of macro- and micro-nutrients has been determined for a number of important agricultural crops (Baligar and Duncan, 1990). Introduction and exploitation of this genotypic variation into adapted cultivars may result in more efficient use of nutrients in plants (Clark and Duncan, 1991). In the case of nitrogen (N), a more efficient uptake may also reduce the nitrate pollution of water supplies. Thus, in an important high nitrogen-requiring crop such as rapeseed (*Brassica napus* L.; Holmes, 1980) this approach may be an effective way of reducing N fertilizer use and N pollution (*eg*. Gerloff, 1963; Vose, 1963; Gabelman, 1976; Clark and Duncan, 1991). However, research on N fertilizer use by *B. napus* has concentrated mainly on agronomic aspects. Research on genetic variation for N uptake and N utilization in *B. napus* has been sporadic and limited in scope. While genotypic differences of *B. napus* have been shown for N uptake and utilization (Yau and Thurling, 1986; Gerath and Schweiger, 1991; Gerath and Balko, 1995), this information has not been used in the development of high yielding *B. napus* germplasm with improved N absorption and/or utilization.

A preliminary screening to determine the existence of useful genotypic variation is a necessary first step when breeding for N efficient *B. napus* genotypes (Devine et al., 1990). A screening technique consisting of several N-levels over a period of several tissue harvests would be ideal for assaying each genotype but, in the context of a breeding program it is impractical and uneconomical. Screening techniques performed using two N levels at one growth stage have been successfully used in the selection of stress tolerant accessions for nutrient stressful environments (see references in Clark, 1990). Screening at two extreme N-levels, a non-stressful and a stressful treatment, would subject plants to conditions in which maximum genotypic potential and stress tolerance of accessions could be assessed (Baker, 1994).

Several factors required for a two N-level screening technique for selecting N efficient *B. napus* germplasm have been examined. The importance of a NH_4/NO_3 mixture for producing maximum plant growth has been demonstrated (Chapter II).

Also, the shoot systems have been shown to be more important than the root systems for assessing N efficiency (Chapter III). Furthermore, the late rosette stage was shown to be appropriate for plant sampling and assaying for N efficiency (Chapter IV) as well as for determining the appropriate N levels for screening (Chapter V).

In this work, genotypic variation for plant growth and N efficiency parameters amongst rapeseed (*Brassica napus* L.) germplasm accessions as assessed by a two N-level screening technique, were examined. An additional objective was to identify N efficiency characteristics and accessions potentially useful for the genetic improvement of N efficiency in *B. napus* germplasm.

VI.2. Materials and Methods

VI.2.a. Plant material and growing conditions

Two N-screenings were done over time on a collection of rapeseed (*Brassica napus* L.) accessions obtained world wide (See Appendix 1 for listing). Each screening included 60 randomly selected accessions. The two screenings were grown under conditions similar to those described earlier (Chapter IV and V) in the greenhouse in 10×10 cm square plastic pots filled with a soil-free medium (Vermiculite + Peat Moss + Sand; 3:3:1 volume basis). Plastic bags sealed at one end were inserted into each pot to prevent nutrient loss through the pot's drainage holes. Two to four seeds were seeded per pot and thinned to one seedling per pot seven days after seeding (DAS).

A modified basal nutrient solution (Taylor and Foy, 1985) was added to each individual pot providing (mmol): K, 1.60; PO_4, 0.40; SO_4, 0.40; Mg, 0.60; Cl, 1.34; Mn, 8×10^{-3}; B, 2.4×10^{-2}; Zn, 2×10^{-3}; Cu, 6×10^{-4}; Na, 8.1×10^{-2}; Mo, 4×10^{-4}; EDTA, 0.04; Fe, 0.04. Iron was supplied as Fe-EDTA prepared from equimolar amounts of $FeCl_3$ and Na_2EDTA. All nutrients were supplied in solution, except for Ca which was supplied as powdered $CaSO_4$, and mixed into the medium. A nitrogen (N) solution consisting of 90% $N\text{-}NO_3$ and 10% $N\text{-}NH_4$ provided two N-treatments (*ie.* 1.5 and 5.1 mmol providing a Low N and a High N level, respectively) selected from a dose response curve of cv. Alto (Fig. IV.1, Chapter IV). The two N levels induced a 50 and 100% growth response of cv. Alto, respectively. An earlier experiment demonstrated the positive response of *B. napus* to N supply when $N\text{-}NO_3$ and $N\text{-}NH_4$ was supplied in a mixture rather than as single ions (Chapter II). Measured volumes of all nutrients, including N-treatments, were added to each individual pot 2 DAS. Plants were watered regularly with distilled water and care was taken to prevent waterlogging.

The two sets of accessions were grown under greenhouse conditions at a temperature of 20-23°C and relative humidity of 60-90%. Natural light was supplemented with 400 watt, high pressure sodium lamps (Sylvania) attached to high intensity discharge fixtures providing a basal average PPFD of 292 ± 17 $\mu mol\ m^{-2}\ s^{-1}$ for 16 h daily at soil height surface.

VI.2.b. Plant growth and N efficiency parameters

Shoots were harvested individually, cut at the base of the cotyledons at 28 DAS. Variables measured included leaf area (LA), number of leaves per plant (LNUM), shoot dry weight (SDW), and percent N content in the shoot (N%). Leaf area was measured on a LI-COR LI3100 Area Meter (LI-COR Inc., Lincoln, Nebraska, USA). Leaf presence on a plant was recorded if the leaf lamina was larger than 0.5 cm. Samples were dried in a forced-air flow-oven until constant weight, weighed and ground in a Wiley mill fitted with a 40 mesh screen (Arthur H. Thomas Co., Scientific Apparatus, Philadelphia, PA, USA). Percent nitrogen was measured by the combustion method using a LECO FP-2000 Nitrogen-Protein analyzer (LECO Corporation, St. Joseph, MI, USA). A homogeneous subsample of approximately 0.7-0.9 g was used to determine percentage N content in the shoot.

Calculated parameters included leaf area ratio (LAR = LA/SDW) and total N absorbed (TOTN = ((((SDW×N%)/100)/14.0)×1000)) (mmol^{-1} N). For the purposes of this study, the parameters used to characterize the efficiency of N acquisition and use by accessions and the efficiency of rate of response to increasing N supply were calculated using the following equations:

Equation 1: Nitrogen use efficiency (NUE) = SDW/TOTN (g mmol^{-1} N)

Equation 2: Efficiency of utilization (EU) = SDW (SDW/TOTN) (g^{-2} mmol^{-1} N)

Equation 3: Physiological efficiency (PE) = ($\text{SDW}_{\text{High N}}$ - $\text{SDW}_{\text{Low N}}$)/($\text{TOTN}_{\text{High N}}$ - $\text{TOTN}_{\text{Low N}}$) (g mmol^{-1} N)

Equation 4: Agronomic efficiency (AE) = ($\text{SDW}_{\text{High N}}$ - $\text{SDW}_{\text{Low N}}$)/net N applied) (g mmol^{-1} N)

Equation 5: Apparent recovery (AR) = (($\text{TOTN}_{\text{High N}}$ - $\text{TOTN}_{\text{Low N}}$)/net N applied)) 100 (%)

where: Low N = 1.5 mmol N; High N = 5.1 mmol N; net N applied 5.1 - 1.5 = 3.6 mmol N. Equation 2 was taken from Siddiqi and Glass (1981) while equations 3, 4 and 5 were taken from Craswell and Godwin (1984). By replacing the SDW term with LA in the equations, efficiency components on a LA basis were obtained. Characterization of the plant growth and N efficiency components in response to N supply in *B. napus* were examined previously (Chapters II, III, IV and V).

VI.2.c. Experimental design and statistical analysis

The experimental design of each screening was a split-plot, consisting of 60 accessions per screening, two N-treatments and six plants per N-treatment for a total of 720 pots per screening. To test whether the two sets of accessions were drawn from the same population, the Kolmogorov-Smirnov two-sample test (K-S test; Siegel, 1956) was performed on the growth and efficiency components. Simple linear correlations among growth and N-efficiency components were performed separately for each screening (Gomez and Gomez, 1984). Analyses were carried out with the statistical software SAS/STATS (SAS Institute, 1992).

VI.3. Results and Discussion

VI.3.a. General observations

A total of 57 accessions from the first screening and 55 accessions from the second screening were included in the final analysis. Some accessions did not germinate while others proved not to be *B. napus* and were eliminated from the analysis. According to the non-parametric K-S test (Siegel, 1956), significant differences for most of the growth and efficiency parameters were observed between the two sets of accessions. This indicated that the two samples were drawn from different populations. Therefore, the data of the two screenings were not pooled for purposes of statistical analysis. Although accessions were randomly selected, the first group contained a large percentage of Canadian accessions while the second group contained a large percentage of Australian and European accessions (see Appendix 1, and Appendix 2). The observed results may indicate the presence of differences in the source gene pools or that the growing conditions were not identical at each screening.

The analysis of genotypic variability for growth and N efficiency parameters of *B. napus* germplasm was examined in a three-step approach. First, the genotypic variation of the plant growth parameters LNUM, SDW, LA and LAR at both N levels was assessed. To indicate whether genotypic variability existed for these growth parameters in a stressed and non-stressed N environment. However, the presence of genotypic variability for plant growth parameters does not necessarily imply the existence of genotypic variation for N efficiency. Second, the genotypic variation of NUE and EU at both N levels, which assesses static N efficiencies, was determined. However, the presence of genotypic NUE or EU variation does not imply the existence of genotypic variation for response rate efficiencies. Third, the genotypic variation for AR, AE and PE which quantifies the response rates to incremental N supply needs to be examined. This was done to indicate whether the genetic variation is the result of differential response rates to increasing N supply. An examination of the three types of responses of *B. napus* accessions to N supply was made to determine if it is possible to make selection based on N-efficiency.

VI.3.b. Visual plant characteristics

Most accessions grown in Low N showed N deficiency symptoms such as leaf chlorosis and/or purplish leaf color as well as leaf senescence when harvested (Marschener, 1995). Leaf shedding was not observed among any of the accessions, thus all the accumulated leaf area was measured at harvest. Also, accessions grown in Low N showed a range of developmental growth stages such as rosette, stem elongation and flowering (Harper, 1973). Accessions grown in High N did not show N deficiency symptoms and exhibited a more uniform rate of development in reaching the late rosette growth stage or stem elongation stage (Harper, 1973). The responses of genotypes grown in Low N were consistent with an N deficient environment in which stress tolerance could be assessed. The absence of deficiency symptoms and the presence of a narrow range of growth stages expressed by accessions grown in High N were consistent with a non-stressful N environment in which genotypic potential could be assessed (Baker, 1994).

VI.3.c. Genotypic variability of growth parameters

Variability among rapeseed genotypes was observed for all growth parameters, namely LNUM, LA, SDW, LAR and TOTN when grown in each N environment (Table VI.1; see Appendix 2 for original data). All accessions responded positively to High N relative to Low N (Table VI.1). It appears that morphological variation is available amongst the accessions screened to be useful for selection in a breeding program. This is the first step in determining the feasibility for breeding N efficient germplasm (Devine et al., 1990). A subsample of genotypes selected from this study and screened under similar experimental conditions confirmed the presence of genotypic variability for plant growth components, such as SDW, amongst this germplasm (Appendix V).

When the accessions were grown in Low N, LA was not correlated with LNUM ($r = 0.03$ and 0.16, for screening 1 and 2, respectively), but when grown in High N there was some significant linear correlation between LA and LNUM ($r = 0.64^{***}$ and 0.27^{*}, for screenings 1 and 2, respectively; *, *** significant at 5% and 0.1%, respectively). In general, variability in leaf expansion was not necessarily explained by an increased LNUM. This indicated that selection for high leaf number may not translate into selection for high leaf area when using this *B. napus* germplasm.

The correlations of LA with SDW for accessions grown in Low N were lower than the correlations of LA with SDW for accessions grown in High N (Table VI.2). This might indicate differential sensitivity to N deficiency between accessions as assessed by LA. Leaf expansion which ceases earlier than SDW is more sensitive than biomass growth to N deficiency (Chapter IV). If there are genotypic differences in sensitivity of leaf expansion to N deficiency relative to shoot biomass growth, the correlation between LA and SDW will not be strong, when grown in a N-deficient environment. This scenario would give rise to a low correlation between LA with SDW when accessions are grown in Low N but, a higher correlation when grown in High N, as seen in this study (Chapter

IV). Thus, this may indicate not only that genotypic variability exists for LA and SDW, but also that sensitivity to N deficiency in terms of LA and SDW varies amongst accessions .

Although there were significant correlations of TOTN with SDW and with LA in both N environments, the correlations were low (Table VI.2). This indicated that the accessions that absorbed the larger N amounts did not necessarily produce the larger SDW or LA. The absence of a close correlation of TOTN with SDW and with LA indicated an absence of a common constant N concentration of the tissue maintained by the accessions. Variability of leaf expansion and shoot biomass production among accessions can not be attributed solely to the total N absorbed. Selection for maximum SDW growth or LA expansion potential based on the absolute total N accumulation did not appear to be justified with this germplasm.

Leaf area ratio (LAR) reflects the leafiness of a plant (Gardner *et al.*, 1985). Differences of LAR among accessions indicate differences for differential allocation of resources to either LA expansion or SDW gain. The higher the LAR, the more is allocated to LA, while the lower LAR, the more is allocated to SDW gain. Leaf area ratio was highly significant but negatively correlated with SDW in both N environments but, although it was correlated with LA the levels were low (Table VI.2). This indicated the existence of variability among genotypes for partitioning of photosynthates between shoot biomass and leaf area expansion.

The growth components considered in this study namely LNUM, SDW, LA, LAR and TOTN are all important growth components of the rosette stage (Harper, 1973) and may be potentially useful selection criteria for increasing N efficiency in *B. napus*. Genotypic variability for LNUM, SDW, LA, TOTN and LAR was demonstrated when accessions were grown in both deficient and sufficient N environment. Exploitation of these characters may be possible. The growth components SDW and LA appear to be the most promising growth characteristics suitable for selection when breeding for N efficient *B. napus* germplasm. Whether these are only morphological differences between accessions and not a result of N efficiency differences, needs to be examined in relation to the amount of N absorbed by the accessions .

VI.3.d. Genotypic differences for static N efficiency components

Nitrogen use efficiency (NUE) and EU (equations 1 and 2, respectively) provide a means of measuring N utilization by plants and a means for making comparisons between accessions to asses N efficiency. Nitrogen use efficiency (NUE) is a measure of growth (*eg*. SDW or LA) relative to the N uptake by the plant, whereas EU emphasizes the contribution of total plant growth (*eg.* SDW or LA) and is expressed as the product of total growth times NUE (Glass, 1990; Siddiqi and Glass, 1981). Both efficiency definitions are static assessments of N efficiency. There were large genotypic variations for both NUE and EU when calculated either on a SDW or on a LA basis, and when determined in both N environments (Table VI.1). As compared to Alto, there were accessions with lower and higher efficiency estimates in both N treatments (Table VI.1). Genotypic variability for both NUE and EU appears to be available for genetic improvement of N efficiency in *B. napus* (Table VI.1). A subsample of genotypes selected from this study and screened under similar experimental conditions confirmed the presence of genotypic variability for static N-efficiency components among this germplasm (Appendix V).

Correlations of both NUE and EU were performed with each component of their equations (Table VI.3, VI.4). That is, correlations of NUE with SDW or LA and TOTN and correlations of EU with SDW or LA and TOTN and NUE (Table VI.3, VI.4). In general, in both screenings and when grown in both N environments, both N efficiencies were highly correlated with either LA or SDW but overall with slightly higher correlations when EU was used in the estimates instead of NUE (Table VI.3). Also, EU was highly correlated with its NUE component in both N environments and when calculated in both LA and SDW (Table VI.4). In contrast, the correlations of both EU and NUE, in either LA or SDW basis, with TOTN and when grown in either N environment were very low (Table VI.3). One exception to this was in the second screening, where the correlation of NUE on a SDW basis grown in Low N was negative and significant with TOTN (Table VI.3). Taken together, these results indicate that genotypic variability in LA and SDW is highly associated with the N utilization by the plants and poorly associated with the total N absorbed by the accessions . Genotypic variation, therefore, of SDW and LA can be explained mostly by the genotypic variation of N utilization and not by the genotypic variation of the total N absorbed.

VI.3.e. Genotypic differences for response-rate N efficiency components

The genotypic rate of response to increasing N fertilizer can be assessed by efficiency definitions such as AE, AR and PE (See equations 3, 4, 5; Craswell et al., 1984). The AR is a measure of how efficient a genotype is at obtaining N from the growing medium, AE is a measure of biomass response per unit of N applied and PE is a measure of efficiency of the rate of response of growth to the uptake of N (Craswell et al., 1984). Agronomic efficiency is the product of PE and AR and an improvement of either PE or AR may result in an improvement of AE (Novoa and Loomis, 1981).

Accessions screened in this study showed wide variation for all three N efficiencies when calculated both on a SDW and on a LA basis (Table VI.5). There were accessions expressing N efficiencies above and below the ones measured for Alto (Table VI.5). This indicated the existence of variation in these germplasms in response to increased N fertilizer supply, which may justify genetic selection for all three N efficiency components. A subsample of genotypes selected from this study and screened under similar experimental conditions confirmed the presence of genotypic variability for response-rate N-efficiency components among this germplasm (Appendix V).

The efficiency of N recovery (AR) by accessions was poorly correlated with the LA and SDW when grown in either of the two N environments (Table VI.6). The most efficient accessions for N recovery, therefore, did not necessarily produce the most SDW biomass or the largest LA.

The correlation patterns of AE and PE on either a SDW or LA basis and in either Low or High N environments were similar (Table VI.6). Both AE and PE had lower correlations with SDW when accessions were grown in Low N as compared to accessions grown in High N (Table VI.6). Also, with the exception of AE of screening 1, AE and PE were not correlated with LA when accessions were grown in Low N, but were significantly correlated with LA when accessions were grown in High N (Table VI.6). The high level of correlation with SDW or LA at sufficient N indicates that accessions tended to be very responsive to increasing N (or very sensitive to deficient N). The lack of complete correlation, however, indicated that some accessions did not follow the same response to N supply.

The correlations of AE and PE with NUE and EU showed different patterns of response when estimated in either LA or SDW basis (Table VI.7). Both, AE and PE, had low correlations with NUE and EU when estimated on a LA basis and grown in Low N but, had high correlations when grown in High N (Table VI.7). In contrast, both AE and PE correlated well with NUE and EU when estimated on a SDW basis and grown in both N environments although, in the Low N environment, the level of correlation was slightly lower (Table VI.7).

The correlations of AE with PE when calculated on a LA basis were significant, but they were not as high as when they were estimated on a SDW basis (Table VI.8). On the other hand, the correlations of AE with AR when calculated on either SDW or LA basis were significant but low (Table VI.8). Likewise, the correlations of PE with AR on either SDW or LA were almost insignificant (Table VI.8). This indicated that the response rates of AE by the accessions were almost exclusively explained by the response rates of PE among all screened accessions . This may imply that it might be difficult to increase AE by improving PE. However, this may only be the case with germplasm used in this study.

It can be argued that the time, effort and cost that went into measuring N content was wasted and that AE_{SDW} which was highly correlated with PE_{SDW}, could have been derived just from SDW data and the calculated differences in N available between the high and low N treatments. However, in the context of a plant breeding program and the fact that N-efficiency in plants is the result of a number of components, this approach may result in the loss of valuable genetic variability.

Caution should be excercise when interpreting the correlations used in this study. The correlation between a primary parameter with derived parameter (eg. SDW vs NUE) or the correlation between derived parameter with a common primary parameter (eg. NUE vs AE) may give rise to "autocorrelation". It is not, however, self evident that "autocorrelaion" will result in a correlation of 1.00 nor that it is of no value. For example, in in Table VI.3 list the correlations of NUE_{SDW} (ie. SDW/TOTN) with both SDW and TOTN. Both sets of correlations give diferent results; high correlations with SDW but low with TOTN. This indicates that SDW is a better predictor of NUE_{SDW} than TOTN in this germplasm. Thus, as a tool for trying to detrminuing with of the primary parametrs could be used when selcting genotypes it can be useful.

VI.4. Conclusions

Crop responsiveness to increasing N fertilizer is important in crop production. Because of the wide range of existing farming practices, large variation of N levels is normally found in cultivated soils. A *B. napus* genotype with high NUE and EU, and high shoot biomass as well as a high leaf expansion, accompanied by a high responsiveness to N increase, is a desirable combination in accessions grown in high input cropping systems in which N loss reduction is desired. Whether those traits can be brought together in a genotype would depend on their level of inheritance and their stability of expression.

VI.5. References

Baker R J 1994 Breeding methods and selection indices for improved tolerance to biotic and abiotic stresses in cool season food legumes. Euphytica 73, 67-72.

Baligar V C and Duncan R R 1990 Crops as enhancers of nutrient use. Academic Press, Inc., San Diego, USA. 574 p.

Clark R B 1990 Physiology of cereals for mineral nutrient uptake, use, and efficiency. *In* Crops as enhancers of nutrient use. Eds. B C Baligar and R R Duncan pp 131-209. Academic Press, San Diego, USA.

Clark R B and Duncan R R 1991 Improvement of plant mineral nutrition through breeding. Field Crops Res. 27, 219-240.

Craswell E T and Godwin D C 1984 The efficiency of nitrogen fertilizers applied to cereals in different climates. Adv. Plant Nutr. 1, 1-55.

Devine T E, Bouton J H and Mabrahtu T 1990 Legume genetics and breeding for stress tolerance and nutrient efficiency. *In* Crops as enhancers of nutrient use. Eds. B C Baligar and R R Duncan pp 211-252. Academic Press, San Diego, USA.

Gabelman W H 1976 Genetic potentials in nitrogen, phosphorus and potassium efficiency. *In* Plant adaptation to mineral stress in problem soils. Ed. M J Wright pp 205-212. Cornell University Press, New York, USA.

Gardner F P, Pearce R B and Mitchell R L 1985 Physiology of crop plants. Iowa State University Press, Ames, USA. 327 p.

Gerath H H and Balko C 1995 The influence of genotype and donor plant on the nitrogen efficiency of DH-lines of winter rape. *In* Proceedings GCIRC 9th international rapeseed congress: Rapeseed today and tomorrow. Cambridge, UK. Vol. 3, pp 813-815.

Gerath H H and Schweiger W 1991 Improvement of the use of nutrients in winter rape. A strategy of economically and ecologically responsible fertilizing. *In* Proceedings GCIRC eight international rapeseed congress. Saskatoon, Canada. Vol 4, 1197-1201.

Gerloff G C 1963 Comparative mineral nutrition in plants. Annu. Rev. Plant Physiol. 14, 699-704.

Glass A D M 1990 Ion absorption and utilization: The cellular level. *In* Crops as enhancers of nutrient use. Eds. V C Baligar and R R Duncan pp 37-64. Academic Press, San Diego, USA.

Gomez K A and Gomez A A 1984 Statistical procedures for agricultural research. John Wiley & Sons, New York, USA. 680 p.

Harper F R 1973 A key to standardize the description of growth stages in turnip rape, *Brassica campestris*. Can. Plant Dis. Surv. 53, 93-95.

Holmes M R J 1980 Nutrition of the oilseed rape crop. Applied Science Publishers Ltd., London, UK. 158 p.

Marschener H 1995 Mineral nutrition of higher plants. 2nd ed. Academic Press, London, UK. 889 p.

Novoa R and Loomis R S 1981 Nitrogen and plant production. Plant Soil 58, 177-204.

SAS Institute 1992 SAS/STAT User's Guide. Version 6. 4th ed. SAS Institute, Cary, USA.

Siddiqi M Y and Glass A D M 1981 Utilization index: a modified approach to the estimation and comparison of nutrient utilization efficiency in plants. J. Plant Nutr. 4, 289-302.

Siegel S 1956 Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Company, New York, USA. 312 p.

Taylor G J and Foy C D 1985 Mechanisms of aluminum tolerance in *Triticum aestivum* (wheat). IV. The role of ammonium and nitrate nutrition. Can. J. Bot. 63, 2181-2186.

Vose P B 1963 Varietal differences in plant nutrition. Herbage Abst. 33, 1-13.

Yau S K and Thurling N 1986 Variation in nitrogen response among spring rape (*Brassica napus*) cultivars and its relationship to nitrogen uptake and utilization. Field Crops Res. 16, 139-155.

Table VI.1. Responses of plant growth and static nitrogen efficiency parameters of two screenings of *B. napus* germplasm when grown in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment.

		1.5 mmol N (deficient)					5.1 mmol N (sufficient)				
†Variable	Screening	Mean	σ^2	Range		cv. Alto	Mean	σ^2	Range		cv. Alto
LNUM	1	6.7	1.72	4.8	14.7	6.8	7.2	1.51	4.8	13.3	8.3
LNUM	2	6.6	0.81	5.2	9.3	7.0	7.6	1.16	6.0	10.3	7.2
SDW	1	0.9	0.01	0.6	1.1	0.93	1.3	0.07	0.6	2.0	1.68
SDW	2	1.1	0.05	0.5	1.6	1.15	1.5	0.15	0.7	2.4	1.62
LA	1	177.1	319.9	145.5	221.6	172.8	328.6	1961.5	186.4	445.1	354.3
LA	2	213.7	474.2	119.2	258.3	191.6	371.3	1515.9	227.8	453.8	349.8
TOTN	1	1.7	0.01	1.5	1.9	1.8	4.4	0.08	2.9	4.8	4.4
TOTN	2	1.7	0.04	1.2	2.0	1.4	4.3	0.10	3.4	4.7	3.7
LAR	1	206.9	615.7	162.2	278.4	186.2	261.0	1158.8	188.9	366.4	210.8
LAR	2	212.5	3008.7	115.5	371.2	166.5	257.5	3546.7	162.9	419.9	216.0
NUE_{SDW}	1	0.52	0.01	0.36	0.65	0.53	0.29	0.01	0.2	0.47	0.39
NUE_{SDW}	2	0.64	0.03	0.31	0.99	0.82	0.36	0.01	0.2	0.56	0.44
NUE_{LA}	1	105.9	97.0	87.0	134.5	98.1	75.5	102.8	61.1	127.0	81.4
NUE_{LA}	2	127.8	273.4	59.5	168.4	136.4	86.8	78.5	50.8	103.1	95.2
EU_{SDW}	1	0.45	0.01	0.22	0.72	0.49	0.40	0.03	0.11	0.95	0.65
EU_{SDW}	2	0.71	0.10	0.17	1.38	0.94	0.58	0.08	0.14	1.36	0.71
EU_{LA} ($\times 10^5$)	1	0.19	127.6	0.13	0.30	0.17	0.25	473.0	0.12	0.57	0.29
EU_{LA} ($\times 10^5$)	2	0.28	257.6	0.07	0.44	0.26	0.33	349.1	0.12	0.46	0.33

† LNUM = leaf number, SDW = shoot dry weight, LA = leaf area, TOTN = total absorbed N, LAR = leaf area ratio, NUE = nitrogen use efficiency, EU = efficiency of utilization.

Table VI.2. Correlation coefficients of plant growth parameters of two screenings of *B. napus* germplasm when grown in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment.

†Variable	Screening	Shoot dry weight (SDW)		Leaf area (LA)	
		1.5 mmol N (deficient)	5.1 mmol N sufficient	1.5 mmol N (deficient)	5.1 mmol N (sufficient)
LA	1	0.48^{***}	0.77^{***}		
LA	2	0.19	0.64^{***}		
TOTN	1	0.30^{*}	0.46^{***}	0.41^{*}	0.44^{***}
TOTN	2	-0.13	0.15	0.23	0.39^{**}
LAR	1	-0.69^{***}	-0.80^{***}	0.28^{*}	-0.27^{*}
LAR	2	-0.88^{***}	-0.90^{***}	0.23	-0.33^{*}

† SDW = shoot dry weight, LA = leaf area, TOTN = total absorbed N, LAR = leaf area ratio.
*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

Table VI.3. Correlation coefficients of plant growth parameters with N efficiency parameters of two screenings of *B. napus* germplasm when grown in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment.

†Variable	Screen	Shoot dry weight (SDW) 1.5 mmol N (deficient)	Shoot dry weight (SDW) 5.1 mmol N (sufficient)	Total absorbed N (TOTN) 1.5 mmol N (deficient)	Total absorbed N (TOTN) 5.1 mmol N (sufficient)
NUE_{SDW}	1	0.89***	0.96***	-0.16	0.21
NUE_{SDW}	2	0.87***	0.96***	-0.59***	-0.11
EU_{SDW}	1	0.97***	0.98***	0.06	0.27*
EU_{SDW}	2	0.95***	0.98***	-0.40**	-0.04
		Leaf area (LA)			
NUE_{LA}	1	0.83***	0.86***	-0.16	-0.06
NUE_{LA}	2	0.50***	0.75***	-0.72***	-0.32*
EU_{LA}	1	0.96***	0.93***	0.14	0.11
EU_{LA}	2	0.82***	0.93***	-0.33*	0.05

†SDW = shoot dry weight, LA = leaf area, NUE = nitrogen use efficiency, EU = efficiency of utilization.

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

Table VI.4. Correlation coefficients of N efficiency parameters of two screenings of *B. napus* germplasm when grown in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment.

		1.5 mmol N (deficient)		5.1 mmol N (sufficient)	
†Variable	Screen	NUE_{SDW}	NUE_{LA}	NUE_{SDW}	NUE_{LA}
EU	1	0.97***	0.95***	0.99***	0.98***
EU	2	0.97***	0.89***	0.99***	0.93***

† NUE = nitrogen use efficiency, EU = efficiency of utilization.

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

Table VI.5. Response-rate nitrogen efficiency parameters of two screenings of *B. napus* germplasm in response to a 3.6 mmol N increment.

†Variable	Screening	Mean	σ^2	Range		cv. Alto
AR	1	74.5	58.0	37.0	82.5	72.0
AR	2	72.2	55.1	44.9	82.2	63.1
AE_{SDW}	1	0.12	0.003	-0.01	0.28	0.21
AE_{SDW}	2	0.13	0.003	0.01	0.24	0.13
AE_{LA}	1	42.1	93.1	11.0	74.2	50.4
AE_{LA}	2	43.8	71.4	22.1	58.7	43.9
PE_{SDW}	1	0.15	0.01	-0.03	0.37	0.29
PE_{SDW}	2	0.18	0.01	0.01	0.34	0.21
PE_{LA}	1	56.8	283.2	29.6	159.2	70.0
PE_{LA}	2	60.6	98.1	31.6	78.9	69.7

† AR = apparent recovery, AE = agronomic efficiency, PE = physiological efficiency

Table VI.6. Correlation coefficients of plant growth parameters with N efficiency parameters of two screenings of *B. napus* germplasm when grown in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment.

†Variable	Screen	1.5 mmol N (deficient)	5.1 mmol N (sufficient)
		Shoot dry weight (SDW)	
AR_{SDW}	1	0.30^{*}	0.44^{***}
AR_{SDW}	2	0.27^{*}	0.36^{*}
AE_{SDW}	1	0.58^{***}	0.95^{***}
AE_{SDW}	2	0.53^{***}	0.86^{***}
PE_{SDW}	1	0.59^{***}	0.94^{***}
PE_{SDW}	2	0.53^{***}	0.85^{***}
		Leaf area (LA)	
AR_{LA}	1	0.31	0.36^{**}
AR_{LA}	2	0.18	0.52^{***}
AE_{LA}	1	0.35^{*}	0.93^{***}
AE_{LA}	2	0.09	0.83^{***}
PE_{LA}	1	0.19	0.75^{***}
PE_{LA}	2	0.01	0.69^{***}

† SDW = shoot dry weight, LA = leaf area, AR = apparent recovery, AE = agronomic efficiency, PE = physiological efficiency.

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

Table VI.7. Correlation coefficients of static N efficiency parameters with response rate N efficiency parameters of two screenings of *B. napus* germplasm when grown in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment.

		Nitrogen use efficiency (NUE)		Efficiency of utilization (EU)	
†Variable	Screen	1.5 mmol N (deficient)	5.1 mmol N (sufficient)	1.5 mmol N (deficient)	5.1 mmol N (sufficient)
		Shoot dry weight basis (SDW)			
AE_{SDW}	1	0.61***	0.91***	0.62***	0.93***
AE_{SDW}	2	0.59***	0.84***	0.59***	0.85***
PE_{SDW}	1	0.61***	0.93***	0.63***	0.94***
PE_{SDW}	2	0.61***	0.86***	0.60***	0.87***
		Leaf area basis (LA)			
AE_{LA}	1	0.31*	0.85***	0.33*	0.90***
AE_{LA}	2	0.31*	0.67***	0.21	0.82***
PE_{LA}	1	0.11	0.93***	0.15	0.91***
PE_{LA}	2	0.27*	0.81***	0.13	0.82***

† SDW = shoot dry weight, LA = leaf area, AE = agronomic efficiency, PE = physiological efficiency.

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

Table VI.8. Correlation coefficients of response-rates N efficiency parameters of two screenings of *B. napus* germplasm in response to a 3.6 mmol N increment.

†Variable	Screen	AE_{SDW}	AE_{LA}	AR	PE_{SDW}
AE_{LA}	1	0.72***			
AE_{LA}	2	0.76***			
AR	1	0.45***	0.30*		
AR	2	0.37**	0.53***		
PE_{SDW}	1	0.99***	0.75***	0.37**	
PE_{SDW}	2	0.99***	0.72***	-0.24	
PE_{LA}	1	0.40**	0.86***	-0.18	0.48***
PE_{LA}	2	0.69***	0.88***	0.06	0.71***

† AR = apparent recovery, AE = agronomic efficiency, PE = physiological efficiency.
*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

CHAPTER VII

Genetic analysis of nitrogen efficiency components of doubled haploid progenies derived from a reciprocal cross of two rapeseed (*Brassica napus* L.) cultivars.

VII.1. Introduction

Among crops grown in temperate zones, rapeseed (*Brassica napus* L.) is one of the highest nitrogen (N) requiring crops (Holmes, 1980). The use of high levels of N fertilizer, particularly NH_4^+-based fertilizers, leads to water pollution and soil acidification. The utilization of improved N-efficient rapeseed cultivars may ameliorate the negative impact of N-fertilizers in the environment (Baligar and Duncan, 1990). The availability of a wide genotypic variation in N uptake and utilization by most plant species indicates that it would be possible to select N efficient germplasm (Graham, 1984; Clark, 1990; Gerloff, 1963; Vose, 1963; Gabelman, 1976; Clark and Duncan, 1991). Although a large body of literature is available regarding physiological N uptake and utilization in crop plants, very little detailed information on the genetics of N uptake and utilization is available for any crop plant, including *B. napus*.

Heritabilities for N uptake have been reported to be low. Broad-sense heritabilities for N uptake measured at anthesis and maturity were low in wheat (*Triticum aestivum* L.; Austin et al., 1977). Narrow-sense heritability estimates for total N uptake were also low in two perennial ryegrass cultivars (*Lolium perenne* L.; Holmes, 1965). Low heritabilities for N uptake were obtained in both solution culture (Holmes, 1967) and field (Rogers and Thomson, 1970) studies with *L. perenne*. However, in a later study with *L. perenne* and *L. multiflorum*, Goodman (1977) reported a high heritability (0.73) for N-uptake. Genetic studies of N utilization in perennial ryegrass (*L. perenne*; Thomson and Rogers, 1970) and tomato (*Lycopersicon esculentum* Mill.; O'Sullivan et al., 1974) indicated that non-additive gene effects were most important and the narrow-sense heritability was low. In *B. napus*, heritabilities for N uptake and utilization obtained by Yau and Thurling (1987) were low and consistent with estimates in most other plant species. If breeding N-efficient *B. napus* germplasm is to become routine, further information regarding the genetics of N uptake and utilization at the whole plant level will be needed.

The physiology and genetics of N uptake and utilization in plants are complex (Marschener, 1995). Before undertaking a mechanistic study of the factors affecting N efficiency in *B. napus*, information regarding the genetics of N-efficiency components at the whole plant level is of value. Doubled haploid (DH) lines derived from biparental crosses offers a powerful tool to study the genetics of quantitative characters such as N efficiency components in plants. DH progenies form an immediate F_∞ generation with all of the advantages that self pollinated populations provide (Snape and Simpson, 1982). Microspore-derived DH lines in *B. napus* are similar to lines derived by the pedigree and single seed descent method (Charne, 1990). The evaluation of DH lines developed from different parents and the comparison of the frequency distributions of the DH populations permit detection of genotypic differences (Kotch et al., 1992). Moreover, the use of third and fourth degree statistics such as skewness (the degree of departure of a distribution from symmetry) and kurtosis (the peakedness of a distribution) allows differences with haploid populations to be detected and genetic control to be inferred (Choo and Reinbergs, 1982; Choo et al., 1982). Therefore, a study of a DH progeny should provide insight into N-efficiency components in *B. napus*. In this study a genetic analysis of N-efficiency components of DH progenies derived from a reciprocal cross of *B. napus* cultivars is presented.

VII.2. Materials and Methods

VII.2.a. Plant material and growing conditions

Microspore-derived doubled haploid (DH) lines were developed from F_1 plants obtained from 12 reciprocal crosses of nine spring-type *B. napus* genotypes selected for their variation in N-efficiency (Appendix 4). From this genetic material the genotypes Andor and Taiwan were selected for this study because they provided a large number of DH lines. These two genotypes have been shown to differ in N efficiency (Appendix 4). Prior to crossing, the two genotypes were self-pollinated twice and their progenies were assumed to be homozygous. Hybridization of F_1 plants was confirmed by morphological observations and the analysis of the erucic acid profile of one of the cotyledons at seed germination initiation (Dorrell and Downey, 1964; Downey and Harvey, 1963). Doubled haploid lines were developed by the procedure outlined by Coventry et al. (1988). All DH lines were advanced to DH_2 to increase the seed supply and eliminate possible residual environmental effects on plant growth. From each cross (ie. Andor × Taiwan (An×Tai) and its reciprocal Taiwan × Andor (Tai×An)), 48 DH lines were randomly selected and used in this study.

Plants were grown in 10×10 cm plastic pots filled with a soil-free medium (Vermiculite + Peat Moss + Sand; 3:3:1 volume basis). Plastic bags sealed at one end were inserted into each pot to prevent nutrient loss through the pot's drainage holes. Two to four seeds were seeded per pot and thinned to one seedling seven days after seeding (DAS). A modified basal nutrient solution (Taylor and Foy, 1985) was added to each individual pot providing (mmol): K, 1.60; PO_4, 0.40; SO_4, 0.40; Mg, 0.60; Cl, 1.34; Mn, 8×10^{-3}; B, 2.4×10^{-2}; Zn, 2×10^{-3}; Cu, 6×10^{-4}; Na, 8.1×10^{-2}; Mo, 4×10^{-4}; EDTA, 0.04; Fe, 0.04. Iron was supplied as Fe-EDTA prepared from equimolar amounts of $FeCl_3$ and Na_2EDTA. All nutrients were supplied in solution, except for Ca which was supplied as powdered $CaSO_4$, and mixed into the growth medium. A nitrogen (N) solution consisting of 90% N-NO_3 and 10% N-NH_4 provided 2 N-treatments of 1.5 and 5.1 mmol providing a Low N and a High N treatment, respectively. Measured volumes of all nutrients, including N-treatments, were added to each individual pot 2 DAS. The plants were watered with distilled water and care was taken to prevent waterlogging. Plants were grown under greenhouse conditions at a temperature of 15-23°C and relative humidity of 50-90%. Natural light was supplemented with 400 watt, high pressure sodium lamps (Sylvania) attached to high intensity discharge fixtures providing a basal average PPFD of 315 ± 20 μmol m^{-2} s^{-1} for 16 hrs daily.

VII.2.b. Measurement of plant growth and N efficiency parameters

At harvest, shoots were cut at the base of the cotyledons 28 DAS. At this time most DH lines were at the rosette stage (Harper, 1973). Variables measured consisted of leaf area (LA), number of leaves per plant (LNUM), shoot dry weight (SDW), and percentage N content in the shoot tissue (N%). Leaf area was measured on a LI-COR LI3100 Area Meter (LI-COR Inc., Lincoln, Nebraska, USA). Leaf presence on a plant was recorded if the leaf lamina was larger than 0.5 cm. Samples were dried to constant weight at 65 °C, weighed and grounded in a Wiley mill fitted with a 40 mesh screen (Arthur H. Thomas Co., Scientific Apparatus, Philadelphia, PA, USA). Percentage nitrogen was measured by the combustion method using a LECO FP-2000 Nitrogen-Protein analyzer (LECO Corporation, St. Joseph, MI, USA). A homogeneous subsample of about 0.7-0.9 g was used to determine percent N content in the shoot tissue.

Parameters calculated consisted of leaf area ratio (LAR = LA/SDW) and total nitrogen absorbed (TOTN = $((((SDW \times N\%)/100)/14.0) \times 1000)$ (mmol N). For purposes of this study, the parameters used to characterize the efficiency of N acquisition and use, and the efficiency of rate of response to increasing N supply, were calculated with the following equations:

Equation 1: Nitrogen use efficiency (NUE) = SDW/TOTN (g mmol^{-1} N)

Equation 2: Efficiency of utilization (EU) = SDW (SDW/TOTN) (g^{-2} mmol^{-1} N)

Equation 3: Physiological efficiency (PE) = $(SDW_{High\ N} - SDW_{Low\ N})/(TOTN_{High\ N} - TOTN_{Low\ N})$ (g mmol^{-1} N)

Equation 4: Agronomic efficiency (AE) = $(SDW_{High\ N} - SDW_{Low\ N})$/net N applied) (g mmol^{-1} N)

Equation 5: Apparent recovery (AR) = $((TOTN_{High\ N} - TOTN_{Low\ N})$/net N applied) 100 (%)

Where: Low N = 1.5 mmol N; High N = 5.1 mmol N; net N applied = 5.1 - 1.5 = 3.6 mmol N. Equation 2 was taken from Siddiqi and Glass (1981), while equations 3, 4 and 5 were taken from Craswell and Godwin (1984). By replacing the SDW term for LA in the equations, efficiency components on a LA basis were obtained. The plant parameters LNUM, SDW, LA, TOTN and LAR which are used as components to calculate the N-efficiencies will be referred henceforth as 'plant parameters'. The N-efficiency definitions described by equations 1 and 2 (ie. NUE and EU) will be henceforth referred to as 'static N efficiencies', since they are calculations based at only one N-treatment. The N efficiency definitions described by equations 3, 4 and 5 will be henceforth referred to as 'response-rate N-efficiencies' since, these efficiencies were calculated as a rate of response to an increased N-level (ie. A 3.6 mmol N increment).

VII.2.c. Experimental design and statistical analysis

The experimental design was a split-plot with 98 genotypes, two N-treatments, three plants bulked per N-treatment and three replications where genotypes were randomly assigned to main plots and N-treatments to sub-plots. The plant genotypes consisted of the two parental lines and the 96 DH lines. The experiment was performed twice (ie. screening #1 and #2). The DH lines sum of squares was partitioned into the two populations AnxTai and TaixAn components and the residuals for each analysis. Error mean squares associated with each of both population variances were compared by Levene's test to confirm their homogeneity (Levene, 1960). Mean squares for both DH populations for each trait within each screening were compared by an F-test using the larger of the two mean squares as the numerator (Snedecor and Cochran, 1980). The means of both populations (ie. AnxTai and TaixAn) and parents were compared by t-test (Steel and Torrie, 1980). The distribution of both DH populations for each trait were compared using the Kolmogorov-Smirnov non-parametric procedure (K-S test, Siegel, 1956). The normality of the distribution of each DH population as well as the genetic differences between populations were assessed using coefficients of skewness and kurtosis (Snedecor and Cochran, 1980). All statistical analysis were carried out with the statistical software SAS/STATS (SAS Institute, 1992).

VII.3. Results

VII.3.a. General observations

The erucic acids profile for Andor, Taiwan and the F_1 were 0%, 14.3% and 8.5%, respectively, indicating that the F1 cross was successfully. Seven DH lines were lost during screening. Four DH lines failed to germinate and three samples were lost during handling of the plant material. Because some DH lines included in the study differed between both screenings, data for each N-efficiency component from each screening and N-treatment were analyzed separately. A total of 44 and 45 lines from the Andor×Tai and Tai×Andor crosses, respectively, were included in the statistical analysis of both screenings. Statistical analyses showed that the error variances associated with both DH progenies in each analysis were homogenous (analysis not shown; Levene, 1960).

There were two main sources of environmental variation for the phenotypic expression of the traits under study. The main source of variability was due to the environmental conditions under which the plants were grown (ie. screenings) while the other was due to the N-treatments. Both sources of variability caused a large effect on the phenotypic expression of all traits under study, as shown by the differential responses exhibited by the traits under both screenings and both N-treatments.

VII.3.b. Responses of plant parameters

Except for LNUM, significant differences between progeny means of reciprocal crosses were shown by all plant parameters however, not all were maintained across screenings (Table VII.1). On average, the progeny mean levels of LNUM, LA, and SDW were greater in screening #2 whereas, LAR was greater in screening #1 (Table VII.1). Except for LNUM, for all plant components, progeny means performance was higher under high-N than under low-N (Table VII.1). The SDW was the only plant parameter showing consistent levels of significance across both N-treatments and screenings. Thus, significant environmental and N-treatments effects were indicated. The LA and SDW mean of the DH lines derived from the Tai×An progeny were consistently higher than the mean of the DH lines derived from the An×Tai progeny under both N treatments and both screenings. Furthermore, the MS variances and errors associated with plant components of both progenies tended to be greater in screening #1 (Table VII.2). Significant variability among DH lines was maintained across screenings for some but not all plant parameters. The LNUM and LA were the only plant components showing significant consistent differences in both screenings indicating differences in the allelic composition of the parental cultivars for LNUM and LA (Table VII.2). Although significant responses were shown by SDW, TOTN and LAR, these values were low and not maintained across environments and N-treatments, indicating an absence of significant differences in the allelic composition among parental cultivars and their progenies for these traits (Table VII.2). Furthermore, significant differences between variances of the progenies for TOTN but not for the other plant parameters (Table VII.2) were shown. A greater variance indicates that more extreme segregants were present in the progeny. In this study, the TOTN for the An×Tai progeny had the larger variances indicating that it had the more extreme segregants for TOTN (Table VII.2). Also, LNUM was the only plant component which did not show significant differences between progenies both across environments and N treatments. For the most part, all MS variances and errors associated with plant components tended to be larger under high N than under low N, indicating differential response of DH lines to N supply (Table VII.2).

The plant components that showed significant differences for either skewness or kurtosis were not maintained across screenings (Table VII.3). In general, the progenies were normally distributed as shown by the absence of skewness and kurtosis in the frequency distributions for most plant components under study (Table VII.3). Detection of non-normality was consistent across screenings except for TOTN of the Tai×An progeny. Furthermore, there was a consistent pattern of skewness and kurtosis under both N treatments and screenings, indicating a strong environmental effect in the progeny distributions. Where non-normality was detected, it was the result of significant differences for skewness between the DH progeny distributions and not of kurtosis. However, in general, significant differences between progeny distributions were maintained across screenings (Table VII.3). For the most part, the K-S test showed differences between the frequency distributions of both progenies of all plant components. This is further evidence of the significant differences in either means and/or variances for these traits as shown earlier (Table VII.1, VII.2). In all instances where significant differences were shown by the K-S test (Table VII.3), it was associated with significant differences between means (Table VII.1) but, not all were associated with significant differences between variances (Table VII.2).

VII.3.c. Responses of the static N-efficiency parameters

The mean performance for the static N efficiency parameters NUE and EU calculated on a SDW or LA basis were tabulated in Table VII.4. There were significant differences between progeny means for reciprocal crosses for static N efficiencies. All but one (ie. NUE_{LA}) were maintained across both screenings (Table VII.4). The progeny mean levels were greater in screening #2 (Table VII.4). Mean performance of the progenies were higher under low N than under high N except for one static N-efficiency component (ie. EU_{LA}, screening #2; Table VII.4). The progeny derived from the Tai×An cross had higher means for all static N-efficiency components under both N treatments and screenings (Table VII.4). Furthermore, the MS variances and error associated with the static N efficiencies of both progenies tended to be greater in screening #2 (Table VII.5). Significant variability among DH lines was maintained across screenings for only NUE_{LA} and EU_{LA}. Significant differences between variances were not maintained across screenings for any static N-efficiency (Table VII.5). In general, the progeny variances tended to be larger under low-N than under high-N (Table VII.5). These N-treatments effects on the phenotypic expression of static N-efficiencies were opposite to the responses shown by plant components (Table VII.2) from which these components were calculated. The variation of static N-efficiencies among DH lines of both progenies were consistently significant for NUE and EU on a LA basis, but not on a SDW basis, across both N-treatments and screenings (Table VII.5). Thus, there appears to be differences in the allelic composition of the parental cultivars for NUE and EU when calculated on a LA basis. The Tai×An progeny produced the wider genotypic variation, indicating that it had greater extreme segregants (Table VII.5). However, almost all static N efficiencies that showed significant differences between progeny distributions were maintained across screenings (Table VII.6). Also, the static N-efficiencies that showed significant differences for skewness or kurtosis were not maintained across screenings (Table VII.6). In general, static N-efficiency components were normally distributed (Table VII.6). Where non-normality was observed, the frequency distributions always indicated positive skewness indicating the presence of a higher proportion of genotypes showing a high level of N-efficiency among the DH lines. The only consistency was the non-normality of NUE_{LA} AND NUE_{SDW} at both DH progeny in screening #2, showing a significant skewness and kurtosis of the progeny distributions. This indicated a higher than expected number of genotypes with low NUE lines. Also, for the most part, the K-S test showed significant differences between the progeny frequencies (Table VII.6). As shown earlier, NUE_{LA} did not show significant differences for either means (Table VII.4) or error variance estimates across N treatments or environments (Table VII.5) which was confirmed by the K-S test. In all other cases where significant differences were shown by the K-S test, the progenies differed in mean and/or variance estimates.

VII.3.d. Responses of response-rate N-efficiency parameters

The AE_{LA} and AR were the only response-rate N-efficiency parameters that showed significant differences between progeny means. These differences were maintained across both screenings (Table VII.7). The progeny mean levels for all response-rate N-efficiencies were greater in screening #2. There were no significant differences between progenies for PE means on either a SDW or LA basis. Furthermore, significant variability among DH lines was not shown for most response-rate N-efficiencies. The two response-rate N-efficiencies that showed significant differences among DH lines and/or between progenies were not maintained across screenings (Table VII.8). Although significant differences were shown by the static N-efficiencies among progenies (Table VII.5) resulting in significant variability among DH lines for the plant components (Table VII.2), these effects did not generally result in significant variabilities among DH lines for response-rate N-efficiencies (Table VII.8). Few response-rate N-efficiency components showed significant differences for variability among DH lines, indicating an absence of genetic variation for these traits in the parental lines. Also, PE_{LA} was the only response-rate N efficiency that showed a significant skewness and kurtosis that were maintained across screenings (Table VII.9). However, the response rates AE_{LA} and AR were the only static N-efficiencies that showed significant differences between progeny distributions both of which were also maintained across screenings (Table VII.9). Where non-normality was detected, the K-S test did not detect any difference between the progeny frequency distribution. PE_{LA} was the only response-rate N-efficiency which showed non-normality in both progenies and screenings (Table VII.9). However, these differences were not detected by the K-S test. Both progenies, therefore, had similar distributions and were negatively skewed, indicating a preponderance of lines with a higher than expected proportion of higher PE_{LA} lines. Also, significant differences for the response rate N efficiencies AE_{LA} and AR between progeny distributions were shown by the K-S test (Table VII.9). This further corroborates the significant differences shown by the progeny means (Table VII.7) but not by the variances (Table VII.8).

VII.4. Discussion

VII.4.a. Genotype × environment interactions

A general understanding of the genetics as well as the environmental effect on the trait is a main concern when breeding for a particular plant trait. Quantitative traits, such as the N-efficiency components in this study, will be normally-distributed in a large sample of DH lines produced from a biparental cross. This expectation is based on the assumptions of random union of gametes during meiosis, and random gamete sampling through haploidy, with no linkage or epistasis, and no differential survival of gametic genotypes (Falconer, 1981). Non-normality may result if these assumptions are violated (Falconer, 1981). However, non-normality may also be the result of the differences in the interaction of the DH lines with the environment in which the traits are measured, since the phenotypic distribution is based on the DH line means, which contain effects due to genotypes, environment, and genotype × environment interaction (Falconer, 1981). By evaluating the material under study in different environments non-normality caused by a G×E interaction can be separated from that which has a significant genetic component (Choo and Reinbergs, 1982). A persistence of non-normality across environments will indicate the presence of a large genetic component while, the presence of non-normality in some environments but absent in others will indicate a non-normality which is environment-specific.

The results of this study indicated large environmental effects on the traits under study. For example, a significant negative skewness of PE_{SDW} of the An×Tai DH progeny in the first screening, but absent in the second screening (Table VII.9), is an example of non-genetic normality. The positive kurtosis for PE_{LA} present in the same DH progeny in both screenings, by contrast, is likely of genetic origin (Table VII.9). Genetically, kurtosis for a quantitative trait can occur when there are few gene differences between the parents, or when some alleles for which the parents differ contribute substantially more to the expression of the trait than do others (Mather and Jinks, 1971; Choo and Reinbergs, 1982). While the precise genetic basis for kurtosis for PE_{LA} cannot be indicated in this case, its existence can be of benefit to the breeder when selection for PE_{LA} is an important selection criterion. A high frequency of N efficient PE_{LA} lines can be obtained through haploid breeding.

These response-rate N-efficiencies reflect differential rates of response of DH lines to increasing N supply. These phenotypic responses can be seen in the significant AE_{DW} and AE_{LA} in screening #2 (Table VII.3). If genetic differences for any of the N-efficiency components were not present in the parents, then, there would not have been any difference between the DH progeny derived from the bi-parental cross.

The significant effect of environment on the performance of the progenies makes it difficult to interpret the data for determining the basis of the genetic responses. A large genotype × environment interaction was shown by the DH lines and its phenotypic expression of the traits in this study. This is not surprising considering that others have found that N uptake and utilization traits have low heritabilities (Yau and Thurling, 1987). However, a strong genotypic component was present for several plant components as expressed by the N-efficiencies. It is important, therefore, to test lines in different environments and N levels when selecting for N-efficiency in plants.

VII.4.b. Differences between DH populations (maternal effects)

The most significant results from this study were the differences shown between DH progenies for several N-efficiency components. The plant components LA expansion and SDW accumulation were strongly influenced by the maternal parent. Furthermore, there was a significant maternal effect on both NUE and EU on a LA and SDW basis. These results clearly indicated a strong maternal effect on the genetics of N efficiency components. However, the strong environment and N-treatment effects on the phenotypic responses of DH lines makes an examination of these components most likely to be controlled by maternal inheritance difficult. Three different classes of maternal effects have been described: cytoplasmic genetic, endosperm nuclear, and maternal phenotypic (Roach and Wulff, 1987). To determine the true nature of the maternal effect, genotypes would have to be tested under a wide range of environments and N treatments. Difficulty arises because a large number of genotypes would need to be tested to select a genotype expressing true maternal inheritance (of genetic origin). This study was not designed to sort out the different classes of maternal effects. Maternal effects in plants have been reported previously for several traits (reviewed by Roach and Wulff, 1987). For example, maternal effects have been shown for seedling growth rate, leaf area and tiller number in *Dactylis* (Parker, 1968a,b), seedling root length and plant weight in *Arabidopsis* (Corey, et al., 1976) and pod length and yield per row in *B. campestris* (Singh and Murty, 1980).

VII.4.c. Efficiency components (genetics)

Most of the genetic differences shown amongst DH lines can be attributed to N-utilization and N-uptake. An important observation was the fact that these variabilities were shown under both N treatments. Contrary to other reports, in this study significant differences in N-utilization were shown at both low-N and high-N levels. For example, cultivars of *B. napus* grown at different N levels were previously shown to have significant differences in N utilization only at a low N level which limited plant growth (Yau and Thurling, 1986). The low heritabilities reported for N utilization in *L. perenne* (Thomson and Rogers, 1970) and *B. napus* (Yau and Thurling, 1987) may reflect the absence of significant genotypic variation under a non-limiting N supply. Because of these observations Yau and Thurling (1986) concluded that there would be little scope for selecting genotypes capable of yielding at current levels under reduced nitrogen inputs. Gerath and Schweiger (1991) however, concluded that selection of improved use of N efficient winter type *B. napus* might be possible.

Our results showed genetic variability is present among *B. napus* lines under N-stress and N-sufficient conditions. This indicated the possibility of selecting lines that are tolerant to N-deficiency as well as lines that are more responsive under sufficient N-levels.

VII.4.d. Selection of N-efficiency parameters

The variability shown for plant components, static N-efficiencies, and in some instances, response-rate N-efficiencies, indicated the presence of genotypic variability for selection in the progenies tested. However, the use of several N-efficiency components and N-levels might not be the most efficient approach. The main criteria for selecting an N-efficiency component and N-treatment to be used in a screening protocol, will depend on the target environment in which the genotype is to be grown. A breeding program involving selection for 'N uptake efficiency' and 'N utilization efficiency' can be divided into two major objectives (Yau and Thurling, 1987; Vose, 1990). The breeder may wish to improve yield in a system in which static levels of N fertilization are maintained (Yau and Thurling, 1987; Vose, 1990). Alternatively, the breeding program could be designed to produce cultivars capable of yielding at current levels under significantly reduced inputs of N. Thus, improved efficiency in N usage by crops, might be accomplished through an increase in yield at static levels of N or through the maintenance of current yield with a reduction in N inputs (Yau and Thurling, 1987; Vose, 1990).

This study has shown that LA and LA-based efficiency components express far more variability than SDW and SDW-based N efficiency components among the DH lines. Earlier I showed LA to be significantly more sensitive than SDW to N deficiency (Chapter IV, Fig. IV.4). Furthermore, a number of reports indicated the high sensitivity of LA to N-deficiency (Ogunlela et al., 1989). The combination of a sensitive plant component to

N supply together with a well defined N efficiency component that will express the definition of the target environment would make N efficiency improvement in *B. napus* possible.

VII.5. Conclusions

The present study indicated that selection of N-efficient *B. napus* germplasm might be possible, but difficult. Nitrogen use efficiency (NUE) appeared to be the N-efficiency component with the most potential for breeding in *B. napus*. Genetic expression of N-efficiency components were expressed for tolerance to N-deficiency levels as well as for maximum productivity under N-sufficiency levels. Furthermore, a strong maternal effect for N-efficiency was determined in this cross. Although a large genetic component is present for many of the N-efficiencies in this study, the strong environmental effect on genotypic expression on the progenies may restrict the full exploitation of this genetic variability. Whether these genetic differences may be observed under field conditions and result in differential yield responses is the appropriate next step to be addressed.

VII.6. References

Austin R B, Ford M A, Edrich J A and Blackwell R D 1977 The nitrogen economy of winter wheat. J. Agric. Sci., Camb. 88, 159-167.

Baligar V C and Duncan R R 1990 Crops as enhancers of nutrient use. Academic Press, Inc., San Diego, USA. 574 p.

Charne D G 1990 Comparative analysis of microspore-derived and conventional inbred populations of spring oilseed rape, *Brassica napus* L. Ph. D. Thesis, University of Guelph. 145 p.

Choo T M and Reinbergs E R 1982 Analysis of skewness and kurtosis for detecting gene interaction in a doubled haploid population. Crop Sci. 22, 231-235.

Choo T M, Reinbergs E R and Park S J 1982 Comparison of frequency distributions of doubled haploid and single seed descent lines in barley. Theor. Appl. Genet. 61, 215-218.

Clark R B 1990 Physiology of cereals for mineral nutrient uptake, use, and efficiency. *In* Crops as enhancers of nutrient use. Eds. V C Baligar and R R Duncan pp 131-209. Academic Press, San Diego, USA.

Clark R B and Duncan R R 1991 Improvement of plant mineral nutrition through breeding. Field Crops Res. 27, 219-240.

Corey L A, Matzinger D F and Cockerham C C 1976 Maternal and reciprocal effects on seedling characters in *Arabidopsis thaliana* (L.) Heynh. Genetics 82, 677-683.

Coventry J, Kott L and Beversdorf W D 1988 Manual for microspore culture technique for *Brassica napus*. Department of Crop Science, Technical Bulletin, OAC Publication 0498. 35 p.

Craswell E T and Godwin D C 1984 The efficiency of nitrogen fertilizers applied to cereals in different climates. Adv. Plant Nutr. 1, 1-55.

Dorrell D C and Downey R K 1964 The inheritance of erucic acid content in rapeseed (*Brassica campestris*). Can. J. Plant Sci. 44, 499-504.

Downey R K and Harvey B L 1963 Methods of breeding for oil and meal quality. Can. J. Plant Sci. 43, 271-275.

Falconer D S 1981 Introduction to quantitative genetics. 2nd ed. Longman Inc, New York, USA. 340 p

Gabelman W H 1976 Genetic potentials in nitrogen, phosphorus and potassium efficiency. *In* Plant adaptation to mineral stress in problem soils. Ed. M J Wright pp 205-212. Cornell University Press, New York, USA.

Gerath H H and Schweiger W 1991 Improvement of the use of nutrients in winter rape. A strategy of economically and ecologically responsible fertilizing. *In* Proceedings GCIRC eight international rapeseed congress. Saskatoon, Canada. Vol 4, 1197-1201.

Gerloff G C 1963 Comparative mineral nutrition in plants. Ann. Rev. Plant Physiol. 14, 699-704.

Goodman P J 1977 Selection for nitrogen responses in *Lolium* Ann. Bot. 41, 243-256.

Graham R D 1984 Breeding for nutritional characteristics in cereals. Adv. Plant Nutr. 1, 57-102.

Harper F R 1973 A key to standardize the description of growth stages in turnip rape, *Brassica campestris*. Can. Plant Dis. Surv. 53, 93-95.

Holmes D P 1965 Nitrogen uptake and distribution. Rep. Welsh Pl. Breed. Stn. for 1964, 28-29.

Holmes D P 1967 Genetic control of nitrogen uptake. Rep. Welsh Pl. Breed. Stn. for 1966, 30-31.

Holmes M R J 1980 Nutrition of the oilseed rape crop. Applied Science Publishers Ltd., London, UK. 158 p.

Kotch G P, Ortiz R and Peloquin S J 1992 Genetic analysis by use of potato haploid populations. Genome 35, 103-108.

Levene H 1960 Robust tests for equality of variances. *In* Contributions to probability and statistics. Eds. I Olkin, S G Ghurye, W Hoeffding, W G Madow and H B Mann pp 278-292. Stanford University Press, Stanford, USA.

Marschener H 1995 Mineral nutrition of higher plants. 2nd ed. Academic Press, London, UK. 889 p.

Mather K and Jinks J L 1971 Biometrical genetics: The study of continuous variation. Cornell University Press, Ithaca, USA. 382 p.

Ogunlela V B, Kullmann A and Geisler G 1989 Leaf growth and chlorophyll content of oilseed rape (Brassica napus L.) as influenced by nitrogen supply. J. Agron. Crop Sci. 163, 73-89.

O'Sullivan J, Gabelman W H and Gerloff G C 1974 Variation in efficiency of nitrogen utilization in tomatoes (*Lycopersicon esculentum* Mill.) grown under nitrogen stress. Am. Soc. Hort. Sci. 99, 543-547.

Parker P F 1968a Genetic variation in diploid *Dactylis*. I. Cross-compatability and seedling characters. Heredity 23, 279-300.

Parker P F 1968b Genetic variation in diploid *Dactylis*. II. Emergence date and some morphological and physiological leaf characters. Heredity 23, 369-386.

Roach D A and Wulff R D 1987 Maternal effects in plants. Ann. Rev. Ecol. Syst. 18, 209-235

Rogers H H and Thomson A J 1970 Aspects of the agronomy and genetics of quality components in a diallel set of progenies of *Lolium perenne*. L. J. Agric. Sci., Camb. 75, 145-158

SAS Institute 1992 SAS/STAT User's Guide. Version 6. 4th ed. SAS Institute, Cary, USA.

Siddiqi M Y and Glass A D M 1981 Utilization index: a modified approach to the estimation and comparison of nutrient utilization efficiency in plants. Journal of Plant Nutrition 4, 289-302.

Siegel S 1956 Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Company, New York, USA. 312 p.

Singh J N and Murty B R 1980 Combining ability and maternal effects in *Brassica campestris* variety yellow sarson. Theor. Appl. Genet. 56, 265-272.

Snape J W and Simpson E 1982 The use of doubled haploid lines for genetical analysis in barley. *In* Barley genetics, Vol IV. Proc. 4th Int. Barley Genet. Symp. pp 704-709. Chapman and Hall, London, UK.

Snedecor G W and Cochran W G 1980 Statistical Methods. 7th ed. The Iowa State University Press, Ames, USA. 507 p.

Steel R G D and Torrie J H 1980 Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed. McGraw-Hill Book Company, New York, USA. 633 p.

Taylor G J and Foy C D 1985 Mechanisms of aluminum tolerance in *Triticum aestivum* (wheat). IV. The role of ammonium and nitrate nutrition. Can. J. Bot. 63, 2181-2186.

Thomson A J and Rogers H H 1970 Recovery and utilization of applied nitrogen by a diallel set of progenies of *Lolium perenne* L. J. Agric. Sci., Camb. 75, 159-167.

Vose P B 1963 Varietal differences in plant nutrition. Herbage. 33, 1-13.

Vose P B 1990 Screening techniques for plant nutrient efficiency: Philosophy and methods. *In* Genetic aspects of plant mineral nutrition. Eds. N El Bassam, M Dambroth and B C Loughman pp 283-289. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Yau S K and Thurling N 1986 Variation in nitrogen response among spring rape (*Brassica napus*) cultivars and its relationship to nitrogen uptake and utilization. Field Crops Research 16, 139-155.

Yau S K and Thurling N 1987 Genetic variation in nitrogen uptake and utilization in spring rape (*Brassica napus* L.) and its exploitation through selection. Plant Breeding 98, 330-338.

Table VII.1. Mean performance of plant components of DH progenies derived from Andor×Taiwan and Taiwan×Andor crosses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment.

	1.5 mmol N (deficient)					5.1 mmol N (sufficient)				
	†LNUM	LA (cm)	SDW (g)	TOTN (mmol)	LAR (cm^2 g^{-1})	LNUM	LA (cm)	SDW (g)	TOTN (mmol)	LAR (cm^2 g^{-1})
Screening #1										
Andor×Taiwan	6.2	173.5	0.71	2.36	255.6	6.2	199.0	0.72	3.23	284.8
Taiwan×Andor	6.3	186.8	0.83	2.34	234.9	6.4	225.0	0.84	3.56	275.6
	ns	**	***	ns	**	ns	***	***	**	ns
Screening #2										
Andor×Taiwan	6.5	181.1	0.93	1.96	199.0	7.0	260.7	1.06	3.99	250.3
Taiwan×Andor	6.5	185.5	1.02	1.86	185.9	7.1	276.5	1.20	4.06	235.5
	ns	ns	***	**	***	ns	**	***	ns	***

† LNUM = leaf number; LA = leaf area; SDW = shoot dry weight; TOTN = total absorbed N; LAR = leaf area ratio
*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; ns = nonsignificant

Table VII.2. Mean squares and errors of plant components associated with the DH progenies derived from Andor×Taiwan and Taiwan×Andor crosses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. Analyzed as a randomized complete block.

	1.5 mmol N (deficient)					5.1 mmol N (sufficient)				
	†LNUM	LA (cm)	SDW (g)	TOTN (mmol)	LAR ($cm^2\ g^{-1}$)	LNUM	LA (cm)	SDW (g)	TOTN (mmol)	LAR ($cm^2\ g^{-1}$)
Screening #1										
Andor×Taiwan	1.30***	1062*	0.049^{ns}	0.089^{ns}	2900^{ns}	1.11***	3087*	0.058^{ns}	0.798^{ns}	1570^{ns}
Taiwan×Andor	1.04***	1415**	0.053*	0.040^{ns}	2419^{ns}	1.40***	2509*	0.048^{ns}	0.432^{ns}	1570*
Error	0.18	663	0.033	0.061	1986	0.23	1622	0.041	0.501	1181
Difference between mean squares	1.25^{ns}	1.33^{ns}	1.07^{ns}	2.20**	1.20^{ns}	1.26^{ns}	1.23^{ns}	1.21^{ns}	1.85*	1.00^{ns}
Screening #2										
Andor×Taiwan	1.45***	425^{ns}	0.018^{ns}	0.070^{ns}	788^{ns}	1.52***	1820**	0.046^{ns}	0.231*	891^{ns}
Taiwan×Andor	1.39***	547*	0.033^{ns}	0.059^{ns}	1089^{ns}	1.43***	1768**	0.048^{ns}	0.125^{ns}	1309^{ns}
Error	0.13	334	0.025	0.059	1060	0.25	899	0.043	0.143	1239
Difference between mean squares	1.04^{ns}	1.28^{ns}	1.84*	1.19^{ns}	1.34^{ns}	1.06^{ns}	1.03^{ns}	1.03^{ns}	1.85*	1.45^{ns}

† LNUM = leaf number; LA = leaf area; SDW = shoot dry weight; TOTN = total absorbed N; LAR = leaf area ratio

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; ns = nonsignificant

Table VII.3. Comparisons of normality and equality of distribution of plant components of DH progenies derived from Andor×Taiwan and Taiwan×Andor croses and screened in a deficient (1.5 mmol) and sufficient (5.1 mmol) N environment.

	Andor×Taiwan			Taiwan×Andor			Kolmogorov-Smirnov test	
†Variable	Skewness	Kurtosis	Normality	Skewness	Kurtosis	Normality	SD	KSa
				1.5 mmol N (deficient)				
Screening #1								
LNUM	0.34^{ns}	0.05^{ns}	Yes	-0.11^{ns}	-0.54^{ns}	Yes	0.11	0.53^{ns}
LA(cm)	0.38^{ns}	-0.53^{ns}	Yes	0.46^{ns}	0.24^{ns}	Yes	0.34	1.60^{*}
SDW(g)	0.22^{ns}	-0.09^{ns}	Yes	-0.08^{ns}	-0.19^{ns}	Yes	0.41	1.92^{**}
TOTN(mmol)	-0.49^{ns}	0.16^{ns}	Yes	-0.58^{ns}	0.01^{ns}	Yes	0.14	0.64^{ns}
LAR(cm^2 g^{-1})	0.99^{**}	3.03^{*}	No	0.69^{ns}	0.10^{ns}	Yes	0.36	1.71^{**}
Screening #2								
LNUM	-0.09^{ns}	-0.27^{ns}	Yes	-0.16^{ns}	-0.38^{ns}	Yes	0.07	0.32^{ns}
LA(cm)	-0.89^{*}	1.60^{ns}	No	0.11^{ns}	-0.07^{ns}	Yes	0.23	1.07^{ns}
SDW(g)	0.22^{ns}	0.77^{ns}	Yes	0.17^{ns}	-0.65^{ns}	Yes	0.46	2.13^{***}
TOTN(mmol)	0.12^{ns}	2.36^{*}	No	1.23^{**}	4.15^{*}	No	0.34	1.60^{*}
LAR(cm^2 g^{-1})	-0.36^{ns}	-0.28^{ns}	Yes	-0.20^{ns}	-0.62^{ns}	Yes	0.36	1.71^{**}
				5.1 mmol N (sufficient)				
Screening #1								
LNUM	0.21^{ns}	-0.26^{ns}	Yes	0.28^{ns}	0.59^{ns}	Yes	0.14	0.64^{ns}
LA(cm)	-0.01^{ns}	-1.02^{ns}	Yes	0.75^{*}	1.53^{ns}	No	0.39	1.81^{**}
SDW(g)	0.15^{ns}	-0.87^{ns}	Yes	0.25^{ns}	1.18^{ns}	Yes	0.39	1.81^{**}
TOTN(mmol)	0.02^{ns}	-0.98^{ns}	Yes	-0.15^{ns}	0.26^{ns}	Yes	0.36	1.71^{**}
LAR(cm^2 g^{-1})	0.40^{ns}	0.29^{ns}	Yes	1.06^{**}	1.78^{ns}	No	0.25	1.17^{ns}
Screening #2								
LNUM	0.39^{ns}	-0.81^{ns}	Yes	0.04^{ns}	-0.02^{ns}	Yes	0.18	0.85^{ns}
LA(cm)	0.13^{ns}	0.94^{ns}	Yes	-0.01^{ns}	0.29^{ns}	Yes	0.34	1.60^{*}
SDW(g)	0.39^{ns}	0.17^{ns}	Yes	0.05^{ns}	-1.26^{ns}	Yes	0.46	2.13^{***}
TOTN(mmol)	-0.15^{ns}	-0.63^{ns}	Yes	-0.79^{*}	1.38^{ns}	No	0.27	1.28^{ns}
LAR(cm^2 g^{-1})	-0.37^{ns}	0.07^{ns}	Yes	0.19^{ns}	-0.64^{ns}	Yes	0.41	1.92^{**}

† LNUM = leaf number; LA = leaf area; SDW = shoot dry weight; TOTN = total absorbed N; LAR = leaf area ratio

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; ns = nonsignificant

Table VII.4. Mean performance of static N efficiency parameters of DH progenies derived from Andor×Taiwan and Taiwan×Andor crosses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment.

	1.5 mmol N (deficient)				5.1 mmol N (sufficient)			
	†NUE_{LA} (cm^2 $mmol^{-1}$)	NUE_{DW} (g $mmol^{-1}$)	EU_{DW} (g^2 $mmol^{-1}$)	EU_{LA} (cm^4 $mmol^{-1}$)	NUE_{LA} (cm^2 $mmol^{-1}$)	NUE_{DW} (g $mmol^{-1}$)	EU_{DW} (g^2 $mmol^{-1}$)	EU_{LA} (cm^4 $mmol^{-1}$)
				Screening #1				
Andor×Taiwan	73.7	0.30	0.23	12949	62.2	0.22	0.16	12391
Taiwan×Andor	79.7	0.35	0.31	15168	63.4	0.23	0.20	14331
	***	***	***	***	ns	***	***	***
				Screening #2				
Andor×Taiwan	93.2	0.48	0.45	16941	65.5	0.27	0.29	17210
Taiwan×Andor	100.3	0.56	0.59	18716	68.4	0.30	0.37	19111
	***	***	***	***	*	***	***	**

† NUE = nitrogen use efficiency; EU = efficiency of utilization.

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; ns = nonsignificant

Table VII.5. Mean squares and errors of static N efficiency parameters associated with the DH progenies derived from Andor×Taiwan and Taiwan×Andor crosses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. Analized as a randomized complete block.

	1.5 mmol N (deficient)				5.1 mmol N (sufficient)			
	†NUE_{LA} ($cm^2\ mmol^{-1}$)	NUE_{DW} ($g\ mmol^{-1}$)	EU_{LA} ($cm^4\ mmol^{-1}$	EU_{DW} ($g^2\ mmol^{-1}$)	NUE_{LA} ($cm^2\ mmol^{-1}$)	NUE_{DW} ($g\ mmol^{-1}$)	EU_{LA} ($cm^4\ mmol^{-1}$)	EU_{DW} ($g^2\ mmol^{-1}$)
				Screening #1				
Andor×Taiwan	128.4**	0.0067*	1.69×10^{7}**	0.018^{ns}	56.8*	0.00049***	1.67×10^{7}**	0.0044^{ns}
Taiwan×Andor	211.7***	0.0085^{ns}	3.51×10^{7}***	0.025^{ns}	63.7***	0.00061^{ns}	2.06×10^{7}***	0.0052^{ns}
Error	61.3	0.0052	1.14×10^{7}	0.014	23.4	0.00036	0.77×10^{7}	0.0036
Difference between mean squares	1.65*	1.27^{ns}	2.08*	1.42^{ns}	1.12^{ns}	1.27^{ns}	1.23^{ns}	1.18^{ns}
				Screening #2				
Andor×Taiwan	127.2**	0.0071^{ns}	1.11×10^{7}*	0.020^{ns}	70.3**	0.0021^{ns}	2.06×10^{7}***	0.0111^{ns}
Taiwan×Andor	170.2*	0.0122^{ns}	1.81×10^{7}**	0.044^{ns}	143.4***	0.0039^{ns}	3.37×10^{7}***	0.0193^{ns}
Error	79.0	0.0115	0.77×10^{7}	0.035	49.1	0.0023	1.10×10^{7}	0.0136
Difference between mean squares	1.34^{ns}	1.72*	1.62^{ns}	2.27**	2.04**	1.86*	1.63^{ns}	1.74*

† NUE = nitrogen use efficiency; EU = efficiency of utilization.

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; ns = nonsignificant

Table VII.6. Comparisons of normality and equality of distribution of static N efficiency components of DH progenies derived from Andor×Taiwan and Taiwan×Andor croses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment.

†Variable	Andor×Taiwan Skewness	 Kurtosis	 Normality	Taiwan×Andor Skewness	 Kurtosis	 Normality	Kolmogorov-Smirnov test SD	 KSa
				1.5 mmol N (deficient)				
Screening #1								
NUE_{LA} ($cm^2\ mmol^{-1}$)	0.08^{ns}	-0.97^{ns}	Yes	0.46^{ns}	-0.59^{ns}	Yes	0.34	1.60^{**}
NUE_{DW} ($g\ mmol^{-1}$)	0.43^{ns}	-0.86^{ns}	Yes	-0.10^{ns}	-0.51^{ns}	Yes	0.46	2.13^{***}
EU_{DW} ($g^2\ mmol^{-1}$)	0.55^{ns}	-0.39^{ns}	Yes	0.29^{ns}	0.02^{ns}	Yes	0.43	2.03^{***}
EU_{LA} ($cm^4\ mmol^{-1}$)	0.34^{ns}	-1.01^{ns}	Yes	0.93^{**}	1.07^{ns}	No	0.30	1.39^{*}
Screening #2								
NUE_{LA} ($cm^2\ mmol^{-1}$)	0.19^{ns}	0.63^{ns}	Yes	0.04^{ns}	-0.54^{ns}	Yes	0.48	2.24^{***}
NUE_{DW} ($g\ mmol^{-1}$)	0.56^{ns}	1.43^{ns}	Yes	0.26^{ns}	-0.42^{ns}	Yes	0.57	2.67^{***}
EU_{DW} ($g^2\ mmol^{-1}$)	0.84^{*}	1.75^{ns}	No	0.22^{ns}	-0.59^{ns}	Yes	0.57	2.67^{***}
EU_{LA} ($cm^4\ mmol^{-1}$)	-0.19^{ns}	0.34^{ns}	Yes	0.23^{ns}	0.05^{ns}	Yes	0.36	1.71^{**}
				5.1 mmol N (sufficient)				
Screening #1								
NUE_{LA} ($cm^2\ mmol^{-1}$)	0.23^{ns}	0.13^{ns}	Yes	0.13^{ns}	-0.62^{ns}	Yes	0.18	0.85^{ns}
NUE_{DW} ($g\ mmol^{-1}$)	0.47^{ns}	0.08^{ns}	Yes	0.26^{ns}	0.19^{ns}	Yes	0.41	1.92^{**}
EU_{DW} ($g^2\ mmol^{-1}$)	0.36^{ns}	-0.68^{ns}	Yes	0.57^{ns}	1.71^{ns}	Yes	0.41	1.92^{**}
EU_{LA} ($cm^4\ mmol^{-1}$)	0.11^{ns}	-1.08^{ns}	Yes	1.06^{ns}	1.92^{*}	No	0.32	1.49^{*}
Screening #2								
NUE_{LA} ($cm^2\ mmol^{-1}$)	1.51^{**}	4.75^{*}	No	1.37^{**}	4.64^{*}	No	0.34	1.60^{*}
NUE_{DW} ($g\ mmol^{-1}$)	1.19^{**}	2.68^{*}	No	1.53^{**}	5.03^{*}	No	0.50	2.35^{***}
EU_{DW} ($g^2\ mmol^{-1}$)	0.90^{**}	0.92^{ns}	No	0.57^{ns}	-0.06^{ns}	Yes	0.41	1.92^{**}
EU_{LA} ($cm^4\ mmol^{-1}$)	0.64^{ns}	1.39^{ns}	Yes	0.49^{ns}	0.38^{ns}	Yes	0.36	1.71^{**}

† NUE = nitrogen use efficiency; EU = efficiency of utilization.

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; ns = nonsignificant

Table VII.7. Mean performance of response-rate N efficiency parameters of DH progenies derived from Andor×Taiwan and Taiwan×Andor crosses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment.

	†AE_{DW} (g $mmol^{-1}$)	AE_{LA} (cm^2 $mmol^{-1}$)	AR (%)	PE_{SDW} (g $mmol^{-1}$)	PE_{LA} (cm^2 $mmol^{-1}$)
			Screening #1		
Andor×Taiwan	0.002	7.11	24.3	-0.09	22.4
Taiwan×Andor	0.003	10.63	33.7	0.04	30.6
	ns	* *	* * *	ns	ns
			Screening #2		
Andor×Taiwan	0.037	22.1	57.1	0.05	36.4
Taiwan×Andor	0.045	25.3	61.7	0.07	39.4
	ns	*	* *	ns	ns

† AE = agronomic efficiency; AR = apparent recovery; PE = physiological efficiency.
*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; ns = nonsignificant

Table VII.8. Mean squares and errors of response-rate N efficiency parameters associated with the DH progenies derived from Andor×Taiwan and Taiwan×Andor crosses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment. Analyzed as a randomized complete block.

	†AE_{DW} (g $mmol^{-1}$)	AE_{LA} (cm^2 $mmol^{-1}$)	AR (%)	PE_{SDW} (g $mmol^{-1}$)	PE_{LA} (cm^2 $mmol^{-1}$)
			Screening #1		
Andor×Taiwan	0.0015^{ns}	91.8^{ns}	0.049^{ns}	0.727^{ns}	4660^{ns}
Taiwan×Andor	0.0016^{ns}	88.2^{ns}	0.032^{ns}	0.344^{ns}	8738^{ns}
Error	0.0016	108.8	0.038	0.552	5962
Difference between mean squares	1.07^{ns}	1.04^{ns}	1.50^{ns}	2.11^{**}	1.88^{*}
			Screening #2		
Andor×Taiwan	0.0027^{*}	121.9^{*}	0.0203^{ns}	0.0087^{ns}	267^{ns}
Taiwan×Andor	0.0026^{ns}	89.5^{ns}	0.0090^{ns}	0.0077^{ns}	238^{ns}
Error	0.0020	71.2	0.0111	0.0079	179
Difference between mean squares	1.06^{ns}	1.36^{ns}	2.25^{**}	1.13^{ns}	1.12^{ns}

† AE = agronomic efficiency; AR = apparent recovery; PE = physiological efficiency.
*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively;
ns = nonsignificant

Table VII.9. Comparisons of normality and equality of distribution of response-rate N efficiency parameters of DH progenies derived from Andor×Taiwan and Taiwan×Andor croses and screened in a deficient (1.5 mmol) and a sufficient (5.1 mmol) N environment.

†Variable	'Andor×Taiwan' Skewness	'Andor×Taiwan' Kurtosis	'Andor×Taiwan' Normality	'Taiwan×Andor' Skewness	'Taiwan×Andor' Kurtosis	'Taiwan×Andor' Normality	Kolmogorov-Smirnov test SD	Kolmogorov-Smirnov test KSa
Screening #1								
AE_{SDW} (g mmol^{-1})	0.03^{ns}	-0.72^{ns}	Yes	-0.11^{ns}	-0.11^{ns}	Yes	0.09	0.43^{ns}
AE_{LA} (cm^2 mmol^{-1})	-0.31^{ns}	-0.64^{ns}	Yes	0.16^{ns}	0.61^{ns}	Yes	0.32	1.49*
AR (%)	-0.12^{ns}	-0.75^{ns}	Yes	-0.27^{ns}	0.31^{ns}	Yes	0.39	1.81**
PE_{SDW} (g mmol^{-1})	-2.33**	6.54**	No	2.12**	12.43**	No	0.16	0.75^{ns}
PE_{LA} (cm^2 mmol^{-1})	-2.26**	7.99**	No	0.96**	9.50**	No	0.21	0.96^{ns}
Screening #2								
AE_{SDW} (g mmol^{-1})	0.15^{ns}	-0.07^{ns}	Yes	-0.40^{ns}	0.94^{ns}	Yes	0.21	0.96^{ns}
AE_{LA} (cm^2 mmol^{-1})	0.01^{ns}	0.94^{ns}	Yes	-0.30^{ns}	0.22^{ns}	Yes	0.34	1.60*
AR (%)	-0.21^{ns}	-0.39^{ns}	Yes	-0.83**	0.68^{ns}	No	0.39	1.81**
PE_{SDW} (g mmol^{-1})	-0.52^{ns}	-0.17^{ns}	Yes	-0.63^{ns}	1.10^{ns}	Yes	0.21	0.96^{ns}
PE_{LA} (cm^2 mmol^{-1})	-1.14*	2.06*	No	-1.06**	2.54**	No	0.21	0.96^{ns}

† AE = agronomic efficiency; AR = apparent recovery; PE = physiological efficiency.

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively;
ns = nonsignificant

Chapter VIII

Summary

VIII.1. General Discussion

Four factors need to be addressed before undertaking a breeding program for a specific edaphic adaptation (Devine, 1982). These four prerequisites are as follows: (1) that techniques are available to assay a plant's response to the particular edaphic stress; (2) that there is useful genetic variation for the plant characteristics required, either in agronomically suited cultivars, in noncultivated forms of the crop species, or in related species; (3) that the character is heritable; and finally (4) that the estimated degree of improvement in adaptation (determined from the range of variation and heritability) is sufficient to be of applied value (Devine, 1982). The results obtained in these studies indicated that the first three prerequisites have been partially fulfilled for the selection of N efficient *B. napus* genotypes.

The studies carried out to develop a screening technique for selecting N efficient *B. napus* genotypes indicated that growth of *B. napus* was maximized when some N was supplied as NH_4^+ (Chapter II). However, there did not appear to be an optimum $NH_4^+:NO_3^-$ ratio for maximum growth. Therefore, when selecting N-efficient *B. napus* genotypes N-treatments containing some NH_4^+ should be used to achieve maximum growth. Furthermore, evidence indicated that the root system had a relatively small influence on the expression of N-efficiency components in shoots of *B. napus* genotypes differing in N-efficiency when grown in a high-N environment (Chapter III). These results give credence to the hypothesis that the shoot system, and not the root system, is the major factor in determining N-efficiency components in *B. napus* genotypes. Therefore, assaying the shoot system to determine N efficiency in *B. napus* genotypes might be an effective way of ranking genotypes.

The studies of the time-course responses to N supply (Chapter IV) and of the effect of N supply on rapeseed growth and N efficiency components late in the rosette stage (Chapter V) provided information for selecting sampling time, N-treatment levels, and N efficiency parameters for screening N efficient germplasm. The results of these studies indicated that a growth period of 25-30 days after seeding will provide sufficient time for the terminal portion of the rosette growth stage to be achieved amongst diverse *B. napus* germplasm sources. Both leaf area and shoot biomass were shown to be useful plant growth components when ranking *B. napus* genotypes for N efficiency. Also, the use of N treatments for a screening technique at or below 1.5 mmol N and at or above 5.1 would provide a stressful and non-stressful N environment when selecting *B. napus* genotypes. These N levels provide a 50 and 100% growth response among genotypes, respectively, and sufficient plant material for analysis. The combined use of these N levels and sampling times provide N environments in which both static and response rate efficiency estimates could be made. Taken as a whole, these studies provide a screening technique for selecting N efficient *B. napus* genotypes indicating that Devine's (1982) first requirement has been fulfilled.

There was wide variation amongst rapeseed genotypes under deficient and sufficient N levels (Chapter VI). This indicated a large variability for both types of N efficiency parameters: namely, static and response-rate N efficiencies. However, the largest range of variability amongst cultivars was shown for the nitrogen use efficiency (NUE) parameter. Therefore, variability among genotypes to acquire and use N, and variability among genotypes to respond to increases in N supply appears to be available for exploitation in a breeding program. Thus, Devine's (1982) second requirement was fulfilled.

The results obtained from screening DH populations derived from parents differing in N efficiency indicated a large genetic component for many of the N efficiency parameters under study (Chapter VII). Nitrogen use efficiency appeared to be the N efficiency parameter with the most potential for fixing in *B. napus*, however, the observed strong environmental effect on genotypic expression of the progeny may restrict the full exploitation of this genetic variability. This indicated that selection of N-efficient *B. napus* germplasm might be possible, but difficult. Thus, Devine's third requirement has only been partially fulfilled.

Much research has been done and reported on the effects of increasing N supply on the growth of crop plants. Recently, an increased volume of literature on the effects of N in crop plants at the molecular level has occurred. It would be inappropriate, however, to conclude that questions concerning the effect of N supply at the whole plant level have been resolved. This is particularly true concerning selection of N efficient plant crop genotypes. The studies presented in this thesis indicated that a number of issues need to be addressed before proceeding with the full implementation of a breeding program for selecting N efficient *B. napus* genotypes. A better understanding of the process at the whole plant level coupled with knowledge forthcoming from molecular biology may provide the necessary base for developing N efficient *B. napus* genotypes that can be useful to farmers.

VIII.2. Conclusions

It can be concluded from this study that: (1) genetic variation for N efficiency parameters amongst germplasm accessions of *B. napus* is available; (2) the genotypic variation might be potentially useful in a breeding program; (3) there is a large genetic component for N efficiency and coupled with a strong maternal effect; (4) a static N efficiency parameter such as NUE is potentially the most promising for crop improvement and (5) the implementation of a breeding program for selecting N efficient *B. napus* is not yet feasible.

VIII.3. References

Devine T E 1982 Genetic fitting of crops to problem soils. p. 149-173. *In* M N Christiansen and C F Lewis (ed.) Breeding plants for less favorable environments. John Wiley and Sons, New York.

Appendix 1

List of rapeseed (*Brassica napus* L.) genotypes used for the screening of N-efficiency components (Chapter VI).

Line	Genotype name	Origin	Line	Genotype name	Origin
1	Torrazo	Italy	112	88-1409K Co-Op/Dark	U of A
2	?	? (Belg)	113	88-1426K Co-Op	U of A
6	Bronowski	Pol	114	88-1409K Co-Op/Pale	U of A
7	BOH 1491	Pol	115	Andor	U of A
10	G.S.L.-I	Ind	116	Altex	U of A
11	Kabulena	Jap	117	Wesreo	Aus
12	Miyauchina	Jap	118	Wesbell	Aus
13	Gogatsuna	Jap	119	Wesway	Aus
14	C. O. Na	Jap	120	Wesroona	Aus
15	Shinkirina	Jap	121	Marnoo	Aus
16	Gulliver	Swe	122	Chikuzen	Jap
17	Osga	Swe	123	Genkai	Jap
18	HJA 81081	Fin	124	Haya	Jap
19	Alku	Fin	125	Chisaya	Jap
24	Gulle	Swe	126	Isuzu	Jap
25	Regia II	Swe	127	Tokiwa	Jap
26	Rigo	Swe	128	Mutu	Jap
27	Nilla	Swe	129	Norin 14	Jap
28	Gulle	Swe	130	Norin 16	Jap
29	Hankkijan Lauri	Fin	131	Norin 17	Jap
44	Petranova	Ger	132	Norin 20	Jap
69	?	? (Ger)	133	China A	Jap
70	Suditalien 1980:5795	? (Ger)	134	Komet	Ger
71	Weiheustephaner	? (Ger)	135	Spaeths	Ger
72	Liho	? (Ger)	136	Erglu	Ger
73	Gulzower	? (Ger)	137	Jumbo	Ger
80	Crusher	Can	138	Tira	Ger
81	Regent	Can	139	Kosa	Ger
82	Tower	Can	140	Janetzkis	Ger
83	Shiralee	Aus	141	Vankka	Finland
84	Hero	Can	142	Kroko	Ger
85	DJ-52	Can	143	Loras	Ger
86	AC Excel	Can	144	Lirasol	Ger
87	OAC Triton	Can	145	Pera	Ger
88	Midas	Can	146	Taiwan	Taiwan
89	Legend	Can	147	?	Bangladesh
90	Profit	Can	148	?	Bangladesh
91	AC Tristar	Can	149	?	Nepal
92	Celebra	Can	150	?	India
93	Bounty	Can	151	Yickadee	Aus
94	Cyclone	Can	152	Barossa	Aus
95	Stallion	Can	154	Iwashiro	? (Jap)
96	Hyola-401	Can	156	Omi	? (Aus)

97	Westar	Can	157	Kongo	? (Aus)
98	Vanguard	Can	159	Liraglu	? (Aus)
100	Global	Can	160	Ukraine A	? (Aus)
101	Delta	Can	161	Ukraine B	? (Aus)
102	Maluka	Aus	163	China D	? (Aus)
103	Alto	U of A	164	CPI91424	China
104	91-1020-1 (N-efficient)	U of A	165	CPI91426	China
105	92-536-5 (High oil/protein)	U of A	166	CPI91429	China
106	91-215-3 (DH increase)	U of A	167	CPI91432	China
107	91-1152- (long pod)	U of A	168	363 (Exchange Nº2)	China
108	91-4211-1 Interspecific	U of A	169	CPI107192	?
109	91-4214-2 Interspecific	U of A	171	Asahi	Kor
110	91-4212-1 Interspecific	U of A	172	Naehan	Kor
111	91-4231-3 Interspecific	U of A	173	Youngsan	Kor

Appendix 2

Appendix 2 lists the original data obtained in both screenings (screening 1 and screening 2) of rapeseed (*Brassica napus* L.) genotypes for N efficiency described in Chapter IV. The numbers 6 and 18, used in the abbreviations at the top of the tables, indicate 1.5 and 5.1 mmol N treatments, respectively. Abbreviations used in the headings are listed bellow.

AELA = agronomic efficiency on a leaf area basis	(cm^2 mmol^{-1} N)
AEDW = agronomic efficiency on a shoot dry weight basis	(g mmol^{-1} N)
AR = apparent recovery	(%)
DW = LDW + STDW = total shoot dry weight per plant	(g)
EULA = efficiency of utilization on a leaf area basis	(cm^4 mmol^{-1} N)
EUDW = efficiency of utilization on a shoot dry weight basis	(g^2 mmol^{-1} N)
LA = leaf area per plant	(cm^2)
LARDW = LA/SDW = leaf area ratio per plant	(cm^2 g^{-1})
LINE = Line number (see Appendix 1)	
LNUM = number of leaves per plant	
NUELA = nitrogen use efficiency on a leaf area basis	(cm mmol^{-1} N)
NUEDW = nitrogen use efficiency on a shoot dry weight basis	(g mmol^{-1} N)
PELA = physiological efficiency on a leaf area basis	(cm^2 mmol^{-1} N)
PEDW = physiological efficiency on a shoot dry weight basis	(g mmol^{-1} N)
TOTN = total N absorbed per plant	(mmol^{-1} N)

Appendix 2 - Screening 1

LINE	LNUM6	LNUM18	LA6	LA18	DW6	DW18	TOTN6	TOTN18	LARDW6	LARDW18
1	6.2	6.5	173.3	306.5	0.8	1.0	1.7	4.5	206.8	291.9
2	5.7	5.7	145.5	259.7	0.6	0.8	1.5	3.9	250.6	315.6
6	6.2	6.0	165.6	259.4	0.8	0.9	1.7	4.2	211.6	278.4
7	5.5	6.3	189.3	337.7	0.8	1.3	1.6	4.4	236.1	266.0
10	6.2	6.8	165.7	269.9	0.6	0.9	1.6	4.0	278.4	309.4
11	4.8	5.7	163.6	299.2	0.8	1.1	1.6	4.5	206.7	276.8
12	6.8	7.7	221.6	376.7	0.9	1.2	1.6	4.4	240.9	318.8
13	5.3	6.0	205.6	353.7	0.9	1.3	1.8	4.6	230.8	273.2
14	5.5	6.3	187.7	317.3	0.8	1.1	1.8	4.4	234.8	294.1
15	6.0	6.7	220.4	352.6	0.9	1.2	1.7	4.6	236.6	296.2
16	6.2	6.8	190.5	339.0	0.9	1.2	1.8	4.5	206.5	278.0
17	6.0	6.3	181.3	305.4	0.8	1.1	1.8	4.4	217.2	286.0
18	7.0	6.4	176.3	262.1	0.7	0.8	1.8	3.9	241.5	320.4
19	7.0	6.6	198.9	378.1	0.9	1.5	1.8	4.5	213.2	248.0
24	6.2	6.5	175.6	317.5	0.9	1.2	1.6	4.6	202.3	271.0
25	6.3	6.7	175.1	324.6	0.7	1.2	1.6	4.5	237.5	272.6
26	6.8	7.3	178.0	321.2	0.7	1.3	1.7	4.5	237.5	256.2
27	6.7	7.3	180.2	328.1	0.9	1.2	1.6	4.4	210.0	264.3
28	6.5	7.0	160.9	325.3	0.8	1.3	1.5	4.4	203.1	249.2
29	6.7	7.0	193.8	356.7	0.9	1.4	1.7	4.4	222.9	252.4
44	5.8	6.3	212.6	369.0	0.9	1.3	1.9	4.8	236.6	277.5
69	6.8	7.5	185.3	336.9	0.9	1.3	1.7	4.5	201.4	251.4
70	6.2	7.3	194.4	362.3	0.9	1.6	1.6	4.4	205.9	225.3
71	6.7	7.3	181.5	344.7	0.8	1.3	1.7	4.6	218.9	259.1
72	7.0	7.5	183.1	360.4	0.9	1.4	1.8	4.5	204.4	254.0
73	6.3	7.0	177.9	333.5	0.8	1.2	1.6	4.4	230.0	267.6
80	6.0	6.6	148.2	283.5	0.9	1.1	1.5	4.3	169.0	249.2
81	5.3	4.8	147.0	186.4	0.6	0.6	1.6	2.9	241.4	323.9
83	7.3	8.0	199.1	364.7	1.1	1.7	1.8	4.5	185.7	217.8
84	7.3	8.0	182.0	375.5	1.1	1.9	1.7	4.5	162.2	197.9
86	7.2	8.2	169.3	324.5	1.0	1.4	1.8	4.6	172.5	224.6
87	6.2	6.5	190.6	340.0	0.9	1.2	1.7	4.5	214.8	287.7
89	7.0	7.3	163.9	314.0	0.9	1.2	1.7	4.4	191.2	260.9
90	6.0	6.3	164.0	252.8	0.8	1.0	1.7	4.1	199.2	262.5
91	6.2	6.3	172.8	303.6	0.8	1.1	1.7	4.3	220.7	287.8
92	6.3	7.2	158.3	311.4	0.9	1.4	1.6	4.2	174.6	216.9
93	6.5	6.5	170.6	321.7	0.9	1.2	1.5	4.3	198.5	276.0
94	7.0	8.0	162.8	311.2	0.8	1.3	1.8	4.5	194.7	235.2
95	5.8	6.3	162.6	299.6	0.7	1.1	1.6	4.4	235.1	283.1
96	7.7	8.0	181.6	378.4	1.0	2.0	1.6	4.2	178.9	188.9
97	6.2	6.5	169.1	325.3	0.9	1.4	1.7	4.3	189.4	233.5
98	6.3	6.8	157.3	272.9	0.8	1.1	1.8	4.4	193.1	256.3
100	6.3	6.4	159.9	298.9	0.9	1.2	1.5	4.0	178.6	246.8
101	5.8	6.3	161.7	314.7	1.0	1.3	1.5	4.3	168.8	246.9
102	7.7	8.8	188.9	399.9	1.0	1.7	1.6	4.3	193.1	234.0
103	6.8	8.3	172.8	354.3	0.9	1.7	1.8	4.4	186.2	210.8
104	7.3	8.5	161.7	336.4	0.9	1.5	1.5	4.3	178.1	219.1
105	6.8	7.5	201.0	423.6	0.9	1.7	1.6	4.4	222.2	252.8
106	14.7	13.3	178.0	445.1	1.0	1.2	1.8	3.5	178.8	366.4
107	7.5	8.5	198.2	390.0	1.0	1.7	1.8	4.7	194.4	233.3
108	6.3	6.8	159.7	269.7	0.8	1.1	1.6	4.0	207.6	251.5
109	8.3	8.3	192.7	352.8	1.0	1.6	1.6	4.3	189.3	218.1
110	6.5	7.0	197.5	364.3	0.9	1.4	1.7	4.4	213.4	263.8
111	8.0	8.5	152.3	306.1	0.8	1.1	1.8	4.6	197.4	288.9
112	8.2	9.2	171.0	350.5	1.0	1.5	1.7	4.5	173.5	226.3
113	8.2	8.7	149.5	329.7	0.9	1.5	1.7	4.5	169.4	217.1
114	7.5	8.5	164.9	329.6	0.8	1.3	1.7	4.3	197.9	247.5

Appendix 2 - Screening 1

LINE	NUEDW6	NUEDW18	NUELA6	NUELA18	EUDW6	EUDW18	EULA6	EULA18
1	0.49	0.23	102.0	68.0	0.41	0.24	17679	20851
2	0.39	0.21	97.5	66.2	0.23	0.17	14183	17184
6	0.47	0.22	98.5	61.1	0.36	0.20	16308	15853
7	0.52	0.29	122.0	76.8	0.41	0.37	23102	25941
10	0.36	0.22	101.0	66.7	0.22	0.19	16735	17993
11	0.50	0.24	104.1	66.8	0.40	0.26	17037	20000
12	0.56	0.27	134.5	85.1	0.51	0.32	29789	32042
13	0.48	0.28	111.8	77.0	0.43	0.36	22994	27230
14	0.46	0.24	107.1	71.4	0.36	0.26	20109	22656
15	0.54	0.26	128.4	77.4	0.51	0.31	28315	27289
16	0.52	0.27	107.1	75.5	0.48	0.33	20404	25582
17	0.47	0.24	101.4	69.8	0.39	0.26	18386	21310
18	0.41	0.21	100.1	66.5	0.30	0.17	17640	17426
19	0.52	0.34	111.8	83.3	0.49	0.51	22240	31492
24	0.54	0.26	109.4	69.4	0.47	0.30	19215	22029
25	0.46	0.26	108.3	72.2	0.34	0.32	18975	23433
26	0.45	0.28	107.0	71.5	0.34	0.35	19035	22961
27	0.53	0.28	112.3	74.0	0.46	0.35	20229	24292
28	0.51	0.29	103.8	73.3	0.40	0.38	16699	23855
29	0.51	0.32	113.5	81.3	0.44	0.46	22000	28987
44	0.48	0.28	113.5	76.7	0.43	0.37	24129	28295
69	0.54	0.29	109.3	74.1	0.50	0.40	20258	24980
70	0.58	0.36	119.6	82.2	0.55	0.59	23259	29770
71	0.48	0.29	105.7	75.4	0.40	0.39	19193	26003
72	0.51	0.31	104.1	79.6	0.46	0.44	19054	28685
73	0.48	0.28	111.1	76.0	0.37	0.35	19755	25363
80	0.57	0.27	95.8	66.2	0.50	0.30	14200	18780
81	0.38	0.20	91.9	63.6	0.23	0.11	13506	11861
83	0.60	0.37	111.1	80.7	0.64	0.62	22127	29432
84	0.65	0.42	104.7	83.5	0.72	0.80	19052	31358
86	0.55	0.32	94.0	71.0	0.53	0.46	15912	23050
87	0.51	0.26	110.2	75.2	0.46	0.31	21005	25559
89	0.51	0.27	96.7	71.2	0.43	0.33	15844	22367
90	0.48	0.24	94.9	61.7	0.39	0.23	15563	15598
91	0.47	0.25	103.0	70.6	0.37	0.26	17798	21431
92	0.57	0.34	99.4	73.4	0.52	0.49	15738	22849
93	0.56	0.27	110.8	74.8	0.48	0.32	18896	24053
94	0.48	0.29	92.7	68.8	0.40	0.39	15095	21398
95	0.43	0.24	102.0	68.2	0.30	0.26	16583	20446
96	0.64	0.47	113.9	89.1	0.65	0.94	20685	33710
97	0.54	0.32	102.4	75.3	0.48	0.45	17313	24509
98	0.47	0.24	89.9	62.2	0.38	0.26	14138	16979
100	0.61	0.30	108.3	74.3	0.54	0.36	17315	22225
101	0.63	0.29	106.0	72.5	0.60	0.37	17148	22812
102	0.60	0.40	116.1	93.6	0.59	0.68	21930	37441
103	0.53	0.39	98.1	81.4	0.49	0.65	16943	28823
104	0.60	0.36	107.5	78.1	0.55	0.55	17385	26270
105	0.58	0.38	128.6	96.2	0.52	0.64	25844	40752
106	0.55	0.35	97.5	127.0	0.54	0.42	17349	56539
107	0.57	0.35	110.2	82.1	0.58	0.59	21843	32016
108	0.48	0.27	98.9	67.0	0.37	0.29	15794	18059
109	0.63	0.38	118.4	81.9	0.64	0.61	22813	28887
110	0.55	0.31	116.3	81.9	0.50	0.43	22962	29845
111	0.44	0.23	87.0	66.5	0.34	0.24	13240	20342
112	0.57	0.34	98.4	77.9	0.56	0.53	16829	27291
113	0.53	0.34	89.2	73.1	0.46	0.51	13338	24103
114	0.49	0.31	97.3	76.9	0.41	0.41	16040	25339

			Appendix 2 - Screening 1		
LINE	AEDW	AELA	AR	PEDW	PELA
1	0.06	37.0	0.78	0.08	47.5
2	0.07	31.7	0.68	0.10	47.0
6	0.04	26.1	0.71	0.06	36.6
7	0.13	41.2	0.79	0.16	52.2
10	0.08	29.0	0.67	0.12	43.3
11	0.08	37.7	0.81	0.10	46.7
12	0.07	43.1	0.77	0.09	55.8
13	0.11	41.1	0.77	0.15	53.7
14	0.08	36.0	0.75	0.10	48.1
15	0.07	36.7	0.79	0.09	46.5
16	0.08	41.2	0.75	0.11	54.7
17	0.06	34.5	0.72	0.09	47.9
18	0.02	23.8	0.61	0.04	39.4
19	0.16	49.8	0.77	0.21	64.9
24	0.08	39.4	0.83	0.10	47.8
25	0.13	41.5	0.80	0.16	51.9
26	0.14	39.8	0.79	0.18	50.6
27	0.11	41.1	0.78	0.14	52.3
28	0.14	45.7	0.80	0.18	57.0
29	0.15	45.2	0.74	0.20	60.7
44	0.12	43.4	0.82	0.15	53.2
69	0.12	42.1	0.79	0.15	53.2
70	0.18	46.6	0.77	0.24	60.3
71	0.14	45.3	0.79	0.18	57.2
72	0.15	49.3	0.77	0.19	64.0
73	0.13	43.2	0.77	0.17	55.9
80	0.07	37.6	0.76	0.10	49.5
81	-0.01	11.0	0.37	-0.02	29.6
83	0.17	46.0	0.76	0.22	60.7
84	0.22	53.8	0.77	0.28	70.2
86	0.13	43.1	0.77	0.17	56.1
87	0.08	41.5	0.78	0.11	53.5
89	0.10	41.7	0.75	0.13	55.3
90	0.04	24.7	0.66	0.06	37.5
91	0.08	36.3	0.73	0.10	49.9
92	0.15	42.5	0.74	0.20	57.7
93	0.08	42.0	0.77	0.11	54.7
94	0.14	41.2	0.77	0.18	53.6
95	0.10	38.1	0.78	0.13	49.0
96	0.27	54.7	0.74	0.37	74.2
97	0.14	43.4	0.74	0.19	58.6
98	0.07	32.1	0.73	0.09	43.9
100	0.09	38.6	0.71	0.12	54.6
101	0.09	42.5	0.78	0.11	54.3
102	0.20	58.6	0.73	0.28	79.8
103	0.21	50.4	0.72	0.29	70.0
104	0.17	48.5	0.78	0.22	62.3
105	0.21	61.8	0.79	0.27	78.4
106	0.06	74.2	0.47	0.13	159.2
107	0.18	53.3	0.82	0.22	65.0
108	0.08	30.5	0.67	0.13	45.6
109	0.17	44.5	0.74	0.22	59.7
110	0.13	46.3	0.76	0.17	60.7
111	0.08	42.7	0.79	0.10	53.9
112	0.16	49.9	0.77	0.20	64.9
113	0.18	50.1	0.79	0.22	63.6
114	0.14	45.7	0.72	0.19	63.5

Appendix 2 - Screening 2

LINE	LNUM6	LNUM18	LA6	LA18	DW6	DW18	TOTN6	TOTN18	LARDW6	LARDW18
103	7.0	7.2	191.6	349.8	1.2	1.6	1.4	3.7	166.5	216.0
115	6.2	6.2	200.0	302.3	0.5	0.7	1.7	3.6	371.2	416.8
116	5.7	6.7	208.7	368.6	0.6	1.0	1.7	4.4	326.8	365.0
117	5.2	6.5	202.6	366.6	0.7	1.2	1.6	4.4	279.0	317.0
118	6.0	6.5	212.3	319.8	0.7	0.9	1.7	4.1	304.9	345.4
119	5.8	6.4	221.2	377.8	0.9	1.2	1.8	4.6	248.3	311.2
120	6.7	7.3	251.4	399.6	0.8	1.2	1.6	4.4	315.4	331.6
121	7.0	7.8	209.4	289.0	0.7	0.7	1.8	3.4	313.6	419.9
122	7.3	9.5	221.6	391.8	0.8	1.2	1.6	4.5	267.7	333.4
123	6.5	7.3	213.4	355.4	0.9	1.2	1.6	4.4	233.4	305.3
124	5.6	6.8	201.5	373.5	0.7	1.3	1.7	4.5	290.4	289.0
125	6.2	6.7	204.7	383.8	1.0	1.5	1.6	4.4	199.5	249.6
126	6.8	9.0	204.7	345.2	1.1	1.4	1.6	4.3	186.9	242.2
127	7.0	8.8	222.6	410.9	0.9	1.4	1.5	4.2	235.4	288.9
128	5.5	6.3	195.8	334.2	1.2	1.3	1.6	4.1	159.5	249.5
129	6.5	8.0	205.4	368.5	1.1	1.7	1.5	4.1	182.6	216.6
130	6.2	6.5	236.8	327.0	1.0	1.2	1.6	4.5	240.6	263.5
131	8.8	10.0	202.3	364.4	1.0	1.4	1.7	4.5	199.8	255.8
132	7.5	9.0	208.2	332.4	1.2	1.3	1.7	4.4	170.8	263.9
133	5.8	6.7	220.5	413.6	1.1	1.8	1.5	4.1	196.6	235.9
134	6.0	7.0	217.8	404.0	1.3	1.9	1.5	4.0	163.8	216.8
135	6.0	7.3	188.8	376.4	1.1	1.9	1.2	3.6	165.5	199.6
136	5.8	7.3	182.7	358.1	0.8	1.4	1.5	4.2	222.6	247.5
137	6.3	7.5	217.2	407.3	1.2	1.7	1.5	4.1	181.7	235.6
138	6.2	7.2	210.0	370.8	1.4	2.0	1.4	3.9	150.5	184.5
139	6.2	7.7	186.1	349.4	1.0	1.5	1.5	4.1	194.6	225.7
140	5.8	6.7	177.2	349.7	1.2	1.9	1.4	4.2	148.6	179.8
141	8.3	10.2	191.7	376.8	1.2	1.9	1.2	3.7	165.1	195.6
142	5.7	6.7	258.3	380.3	1.1	1.7	1.5	4.2	234.4	224.5
143	6.3	7.7	200.2	382.9	1.1	1.8	1.4	4.2	176.8	215.4
144	5.8	6.3	225.7	395.4	1.2	1.8	1.8	4.5	191.7	216.3
145	7.0	8.0	242.7	453.8	1.5	2.4	1.8	4.5	158.0	188.6
146	7.2	7.5	243.2	412.8	1.6	2.4	1.8	4.3	155.2	169.6
147	5.8	6.3	228.6	389.4	1.1	1.6	1.8	4.5	201.5	237.9
148	7.0	8.0	216.5	406.2	1.3	2.1	1.7	4.4	163.7	193.9
149	.	.	119.2	227.8	1.0	1.4	2.0	4.5	115.5	162.9
150	7.0	8.5	221.1	391.8	1.3	1.8	1.8	4.4	167.0	212.7
151	8.8	9.2	228.8	403.2	1.3	1.9	1.8	4.2	172.0	217.2
152	7.7	8.8	232.8	414.0	1.5	1.8	1.7	4.2	160.4	225.1
154	7.7	9.0	223.7	421.2	1.3	2.1	1.8	4.7	170.3	203.4
156	7.2	8.3	221.5	383.7	1.1	1.6	1.9	4.7	209.3	236.9
157	9.3	10.3	212.4	377.2	1.1	1.5	1.8	4.6	198.9	245.4
159	5.7	7.3	208.9	403.7	1.2	2.0	1.8	4.5	169.4	199.4
160	6.3	6.7	211.9	353.4	1.2	1.5	1.7	4.7	176.8	228.3
161	7.2	8.2	229.1	379.5	1.1	1.8	2.0	4.5	199.8	212.3
163	6.3	7.5	225.3	402.0	1.2	1.6	1.8	4.5	187.7	247.3
164	6.7	6.8	206.1	323.8	0.8	1.1	1.8	4.0	245.0	303.2
165	6.3	7.0	216.5	330.8	1.0	1.1	1.9	4.2	213.0	299.7
166	7.3	8.3	254.9	369.2	0.9	1.0	2.0	4.4	273.6	361.4
167	5.8	6.0	196.5	290.1	0.6	0.9	2.0	3.8	314.4	334.1
168	6.3	7.2	220.1	404.2	1.1	1.7	1.8	4.7	198.5	243.5
169	7.8	7.7	215.8	379.6	1.1	1.3	2.0	4.5	204.9	300.7
171	6.2	6.7	228.2	394.6	1.0	1.5	1.9	4.4	232.7	271.4
172	5.5	6.8	217.1	400.7	0.9	1.3	1.9	4.7	239.6	304.6
173	6.5	7.3	241.6	384.6	0.9	1.3	1.9	4.7	276.9	307.3

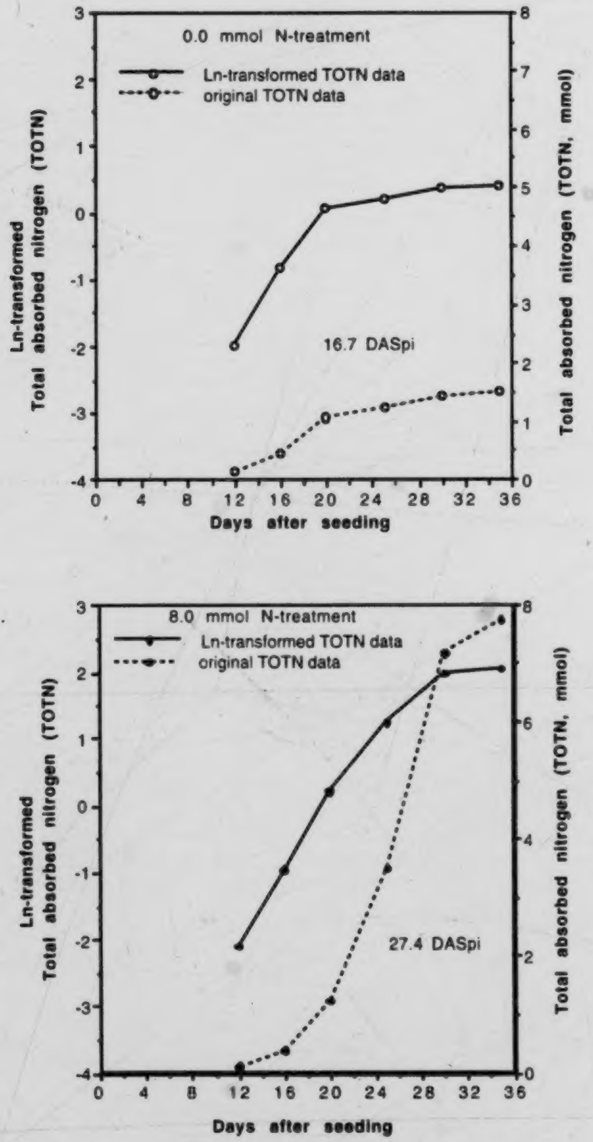
Appendix 2 - Screening 2

NUEDW6	NUEDW18	NUELA6	NUELA18	EUDW6	EUDW18	EULA6	EULA18
0.82	0.44	136.4	95.2	0.94	0.71	26123	33280
0.31	0.20	114.8	84.1	0.17	0.15	22949	25425
0.38	0.23	123.4	84.7	0.24	0.23	25746	31206
0.44	0.27	123.7	84.2	0.32	0.31	25059	30887
0.41	0.23	126.0	78.4	0.29	0.21	26748	25077
0.51	0.26	125.5	81.3	0.45	0.32	27768	30722
0.49	0.27	154.5	89.8	0.39	0.33	38829	35893
0.38	0.20	118.4	85.4	0.25	0.14	24791	24674
0.51	0.26	135.9	87.3	0.42	0.31	30110	34198
0.57	0.26	132.9	80.2	0.52	0.31	28350	28515
0.40	0.29	116.2	83.3	0.28	0.37	23422	31108
0.66	0.35	131.2	86.5	0.67	0.53	26856	33219
0.68	0.33	126.5	81.0	0.74	0.48	25889	27946
0.63	0.34	147.2	97.0	0.59	0.48	32754	39867
0.75	0.33	118.8	81.4	0.91	0.44	23259	27220
0.73	0.42	132.6	90.6	0.82	0.71	27224	33366
0.61	0.28	147.8	73.4	0.60	0.35	35001	23992
0.60	0.32	120.6	80.7	0.61	0.45	24396	29401
0.73	0.28	124.6	74.8	0.89	0.36	25947	24866
0.73	0.43	144.1	101.0	0.82	0.75	31778	41781
0.91	0.47	149.0	100.9	1.21	0.87	32457	40753
0.96	0.52	158.8	103.1	1.09	0.97	29969	38819
0.55	0.34	121.8	84.8	0.45	0.50	22258	30357
0.80	0.42	144.6	98.5	0.95	0.72	31418	40122
0.99	0.52	148.5	95.4	1.38	1.04	31181	35358
0.65	0.38	126.8	85.0	0.62	0.58	23593	29694
0.83	0.47	123.9	84.0	0.99	0.91	21947	29360
0.97	0.52	160.6	101.9	1.13	1.00	30785	38381
0.72	0.40	168.4	90.9	0.79	0.69	43482	34560
0.80	0.43	140.7	92.3	0.90	0.76	28165	35327
0.67	0.41	127.9	88.1	0.79	0.74	28859	34824
0.85	0.54	134.1	101.2	1.30	1.29	32545	45927
0.88	0.56	135.9	95.0	1.37	1.36	33043	39229
0.64	0.36	128.9	86.3	0.73	0.59	29465	33619
0.78	0.48	127.4	92.7	1.03	1.00	27584	37641
0.51	0.31	59.5	50.8	0.53	0.44	7092	11575
0.73	0.42	121.8	89.2	0.97	0.77	26940	34943
0.74	0.44	126.8	95.7	0.98	0.82	29004	38584
0.84	0.43	135.4	97.9	1.22	0.80	31513	40539
0.72	0.44	122.3	90.4	0.94	0.92	27363	38094
0.56	0.34	118.2	81.3	0.60	0.56	26179	31203
0.60	0.34	120.1	82.4	0.64	0.52	25507	31093
0.69	0.45	117.5	89.0	0.86	0.90	24534	35915
0.69	0.33	122.6	75.4	0.83	0.51	25970	26652
0.59	0.40	117.1	84.2	0.67	0.71	26824	31968
0.68	0.36	127.0	89.7	0.81	0.59	28611	36068
0.46	0.26	112.9	80.0	0.39	0.28	23270	25896
0.55	0.26	116.6	79.2	0.56	0.29	25230	26204
0.47	0.23	128.6	83.5	0.44	0.24	32782	30834
0.32	0.23	100.1	77.1	0.20	0.20	19663	22367
0.61	0.35	121.4	85.7	0.68	0.58	26718	34639
0.52	0.28	106.3	84.9	0.55	0.36	22932	32227
0.51	0.33	118.5	90.1	0.50	0.48	27047	35552
0.48	0.28	115.3	85.7	0.44	0.37	25037	34334
0.45	0.27	125.5	82.6	0.40	0.34	30330	31767

		Appendix 2 - Screening 2		
AEDW	AELA	AR	PEDW	PELA
0.13	43.9	0.63	0.21	69.7
0.05	28.4	0.51	0.10	55.3
0.10	44.4	0.74	0.14	60.1
0.12	45.6	0.75	0.16	60.4
0.06	29.9	0.66	0.10	44.9
0.09	43.5	0.80	0.11	54.3
0.11	41.2	0.78	0.14	52.5
0.01	22.1	0.45	0.01	49.2
0.10	47.3	0.79	0.12	59.6
0.07	39.4	0.78	0.09	50.3
0.17	47.8	0.76	0.22	62.5
0.14	49.8	0.80	0.18	62.3
0.09	39.0	0.73	0.12	53.1
0.13	52.3	0.76	0.18	69.2
0.03	38.4	0.68	0.05	56.4
0.16	45.3	0.70	0.23	64.7
0.07	25.0	0.79	0.09	31.6
0.11	45.0	0.79	0.15	57.1
0.01	34.5	0.77	0.01	44.8
0.18	53.6	0.71	0.25	75.3
0.15	51.7	0.71	0.21	73.2
0.21	52.1	0.68	0.30	76.3
0.17	48.7	0.76	0.23	64.4
0.15	52.8	0.73	0.20	72.2
0.17	44.7	0.69	0.25	65.0
0.16	45.4	0.73	0.22	61.8
0.21	47.9	0.76	0.28	63.1
0.21	51.4	0.70	0.31	73.9
0.16	33.9	0.74	0.22	46.0
0.18	50.8	0.76	0.24	67.0
0.18	47.1	0.76	0.24	62.3
0.24	58.7	0.74	0.33	78.9
0.24	47.1	0.71	0.34	66.4
0.14	44.7	0.76	0.18	58.8
0.21	52.7	0.75	0.29	70.7
0.10	30.1	0.69	0.15	43.8
0.14	47.4	0.72	0.20	66.2
0.15	48.5	0.67	0.22	72.4
0.11	50.3	0.70	0.15	72.3
0.21	54.8	0.79	0.27	69.8
0.16	45.1	0.79	0.20	57.0
0.13	45.8	0.78	0.17	58.7
0.22	54.1	0.77	0.29	70.6
0.10	39.3	0.82	0.12	47.9
0.18	41.8	0.71	0.25	59.0
0.12	49.1	0.75	0.16	65.3
0.06	32.7	0.62	0.10	52.9
0.02	31.8	0.64	0.04	49.3
0.02	31.7	0.68	0.04	46.9
0.07	26.0	0.50	0.14	52.0
0.15	51.1	0.81	0.19	63.4
0.06	45.5	0.68	0.09	67.1
0.13	46.2	0.68	0.19	67.8
0.11	51.0	0.78	0.15	65.7
0.11	39.7	0.76	0.14	52.4

Appendix 3

Comparisons between ln-transformed and non-transformed TOTN data selected from Chapter IV (Fig. IV.2a).



Appendix 4

Pairwise comparisons between genotypes used in the reciprocal crosses to develop DH lines (Chapter VII). Data were abstracted from Chapter VI (see Appendix 2 for data). Significance differences between parents were determined by the half-normal plotting technique. The absolute value from the difference between means is compared to 3σ. The estimated "σ" is an estimate of experimental error as derived by the normal plotting technique (Milliken and Johnson, 1989)¶.

Crosses		Nitrogen efficiencies		
Line Nº	Line names	Physiological efficiency	Agronomic efficiency	Apparent recovery
6 × 102	Bronowski × Maluka	sig†	ns	ns
6 × 105	Bronowski × 92-536-5	sig	sig	ns
6 × 107	Bronowski × 91-1152-	ns	ns	sig
6 × 103	Bronowski × Alto	sig	ns	ns
146 × 165	Taiwan × CPI91426	sig	sig	ns
115 × 146	Andor × Taiwan	ns	sig	sig
115 × 144	Andor × Lirasol	ns	ns	sig
166 × 145	CPI91429 × Pera	sig	sig	ns
166 × 148	CPI91429 × ?	sig	sig	ns
166 × 146	CPI91429 × Taiwan	sig	sig	sig
166 × 144	CPI91429 × Lirasol	ns	ns	sig
166 × 160	CPI91429 × Ukraine A	ns	ns	sig

† sig: significantly diferent, ns: not significant.

¶ Milliken G A and D E Johnson 1989 Analysis of Messy Data. Vol. 2. Nonreplicated Experiments. Van Nostrand Reinhold, New York, USA. 199 p.

Appendix V

Table Appendix 5 (Page 149) lists the result of responses of plant growth and nitrogen efficiency components of rapeseed (*Brassica napus* L.) when grown in a deficient (1.5 mmol) and a sufficient (5.1 mmol) nitrogen environment. The lines were a subsample of genotypes taken from the lines screened in Chapter VI. The growing conditions, plant measurements and components calculations were the same as described in Materials and Methods of Chapter VI. The experimental design was a split-plot with four replications.

Table Appendix 5. Responses of plant growth and nitrogen efficiency components of rapeseed (*Brassica napus* L.) when grown in a deficient (1.5 mmol) and a sufficient (5.1 mmol) nitrogen environments.

	1.5 mmol N treatment		5.1 mmol N treatment			
Line number	Shoot dry weight	Nitrogen use efficiency	Shoot dry weight	Nitrogen use efficiency	Agronomic efficiency	Physiological efficiency
145D7	1.68 a	0.85 a	2.5 a-c	0.52 a-c	0.23 abc	0.29 abc
166D3	1.6 ab	0.86 a	2.35 a-e	0.5 a-d	0.21 abc	0.26 abc
166D14	1.55 a-c	0.83 ab	2.62 a	0.56 a	0.3 ab	0.38 a
144D5	1.52 a-d	0.78 a-d	2.21 c-f	0.47 a-e	0.19 bc	0.25 abc
103B52	1.48 b-e	0.74 b-g	2.12 c-f	0.43 c-e	0.18 c	0.22 c
166D5	1.47 b-e	0.79 a-c	2.2 c-f	0.48 a-e	0.2 bc	0.27 abc
115D10	1.46 b-e	0.76 a-f	2.61 ab	0.53 ab	0.31 a	0.37 ab
166D11	1.45 b-f	0.77 a-e	2.37 a-d	0.5 a-d	0.26 abc	0.32 abc
148D6	1.43 b-g	0.74 b-g	2.32 a-f	0.48 a-e	0.25 abc	0.31 abc
165D13	1.42 c-g	0.78 a-d	2.04 d-f	0.47 a-e	0.17 c	0.25 bc
107A9	1.39 c-g	0.66 fg	2.01 d-f	0.39 e	0.17 c	0.21 c
102A5	1.38 c-g	0.64 g	2.16 c-f	0.43 c-e	0.22 abc	0.28 abc
146D13	1.35 d-g	0.7 c-g	1.98 ef	0.41 de	0.17 c	0.21 c
105A4	1.34 d-g	0.7 c-g	2.09 d-f	0.43 c-e	0.21 abc	0.26 abc
6A2	1.33 d-g	0.68 d-g	2.04 d-f	0.44 c-e	0.2 bc	0.26 abc
160D12	1.32 e-g	0.67 e-g	2.02 d-f	0.41 de	0.19 bc	0.24 c
BOSS	1.26 fg	0.67 e-g	2.24 b-f	0.46 b-e	0.27 abc	0.33 abc
144D6	1.26 g	0.68 c-g	1.95 f	0.42 de	0.19 bc	0.25 bc

† Means followed by the same letter are not significantly different at the 5% level according to Duncans multiple range test.